Piezo-Based Scanning Stages for Precise Sample Positioning and Measurement: Ultra-High-Resolution Microscopy in a Modular System

In life sciences, chemical-pharmaceutical analyses or modern material sciences, the optical resolution and information content of classical microscopy methods are no longer sufficient. In order to obtain the most comprehensive information on a sample, modular, high-resolution microscope systems open up interesting opportunities, since different microscopy methods can be used either individually or in combination. As high-precision and dynamic sample positioning is indispensable in most application areas, piezo-based scanning stages are a good solution. Thanks to their compact design, they can easily be integrated in microscopes.

Fig. 1
The modular microscopy systems from WITec make it possible to combine a confocal Raman microscope with atomic force microscopy (AFM) (Source: WITec/PI Physik Instrumente)

The ultra-high-resolution microscopy systems from WITec have a modular structure (Fig. 1). This makes it possible to combine a confocal Raman microscope with atomic force microscopy (ATM), if required. The same device can then provide and link molecular Raman and structural AFM information from the same sample region. For high-resolution optical information, the microscope can also be equipped with SNOM (scanning near field optical microscopy). As a result, precise optical, topographical and molecular analyses are

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Physik Instrumente (PI)
GmbH & Co. KG
76228 Karlsruhe
Germany

www.pi.ws

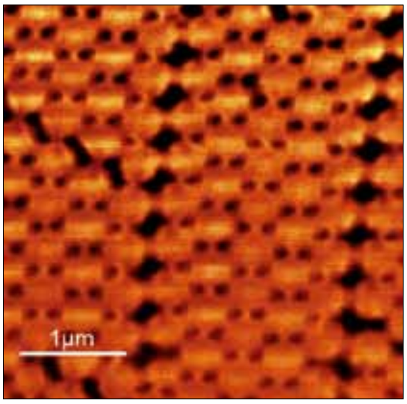

Fig. 2
For high-resolution optical information, the microscope can also be equipped with SNOM (scanning near field optical microscopy). With SNOM, far smaller structures can be shown than with conventional microscope technology (Source: WITec/PI Physik Instrumente)

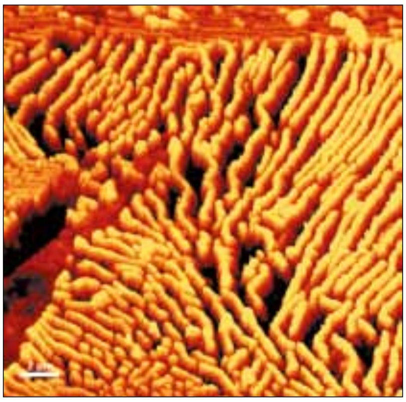

Fig. 3
Atomic force microscopy (AFM) provides precise information on the surface topology (Source: WITec/PI Physik Instrumente)

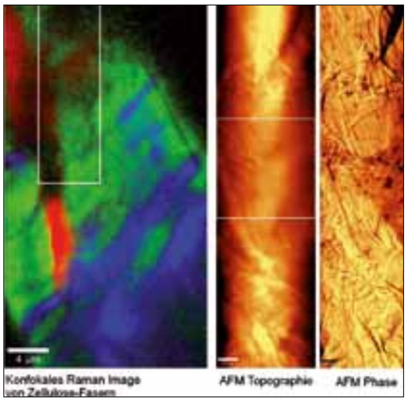

Fig. 4
The Raman image of a paper surface. Raman spectra act like a specific fingerprint for each type of molecule, so that chemical components present in a sample can be identified for each pixel and their distribution in the sample can be shown (Source: WITec/PI Physik Instrumente)

possible, just as required by the respective application. This opens up opportunities for diverse of application areas. The application spectrum of modular high-precision microscopes ranges from pharmaceutical research and live cell analyses, through nanophotonics, forensics up to analyses in photovoltaic or semiconductor technology.

Close-ups below the diffraction limit

With conventional microscopy technology, the position resolution is limited to half of its wavelength due to diffraction effects on the objective. In contrast, scanning near-field optical microscopy can show far smaller structures. Here, a glass fiber couples laser light into a hollow measuring tip. This light emerges through a tiny opening at the tip with a diameter of less than 100 nm. If the opening of the measuring tip is brought closely to the sample surface, a spot far below the diffraction limit of classical microscopy can be illuminated. Depending on the geometry of the measuring tip and its opening, a lateral position resolution of up to around 60 nm can be attained, while with confocal (light) microscopy the value would be between 200 to 300 nm.
For scanning the sample point by point, it is moved under the measuring tip by a piezo-driven, high-resolution scanning stage. At each position, the camera integrated in the microscope records the incoming light intensity and saves this value together with the position information, which is used to create the image. The position resolution and accuracy of the image depend on the positioning accuracy and stability of the scanning stage as well as the optical and mechanical components of the microscope.

Information on the surface topology

SNOM simultaneously provides information on the surface topology: Since the distance between the measuring tip and the surface has to be kept constant and practically every surface has a certain roughness, the position of the sample must be readjusted in the Z direction. This readjustment is carried out by the scanning stage and provides topological information additionally to the optical SNOM image.
In the case of the AFM method, the measuring tip is also moved over the sample surface line by line in a defined grid. Forces are measured between a very thin measuring tip and the surface of the object, which then provides information on the topology of the surface. In addition, sample characteristics such as adhesion, stiffness or viscosity can be determined. The lateral resolution is 10 nm and below. The position of the sample is readjusted in the direction of the Z axis here as well. The variation of the Z position together with the relevant X and Y coordinates for the spatial resolution then provide high-precision topology information on the samples (Fig. 3).

The chemical fingerprint

Raman microscopy is based on a confocal, optical microscope combined with a Raman spectrometer. In the case of a confocal system, apertures are used to suppress light outside of the focal plane of the microscope. In this way, only light information from the focal plane is transferred to the spectrometer. In the spectrometer, this light is spectrally separated and detected. The sample is scanned point by point and line by line. The lateral resolution is approximately 200 nm with green excitation light. During the measurement, a complete Raman spectrum is recorded for each pixel. These Raman spectra act like a specific fingerprint for each type of molecule, so that the chemical components of a sample can be identified for each pixel and their distribution in the sample can be shown (Fig. 4).
Combining Raman imaging with AFM yields topographical information with high spatial resolution as well as molecular information on the sample surface. Since the corresponding images are recorded in succession (Fig. 5), the requirements for the scanning stage are very high. Any drift would distort the correlation between the two images. Precise positioning in all three axes is indispensable for the accuracy of the image.

Positioning with a very high position resolution and dynamics

Since the positioning system used for scanning provides the spatial resolution, its resolution must be in the sub-nanometer range. At the same time, the requirements for the dynamics are high: The faster the topography tracking in the Z direction, the

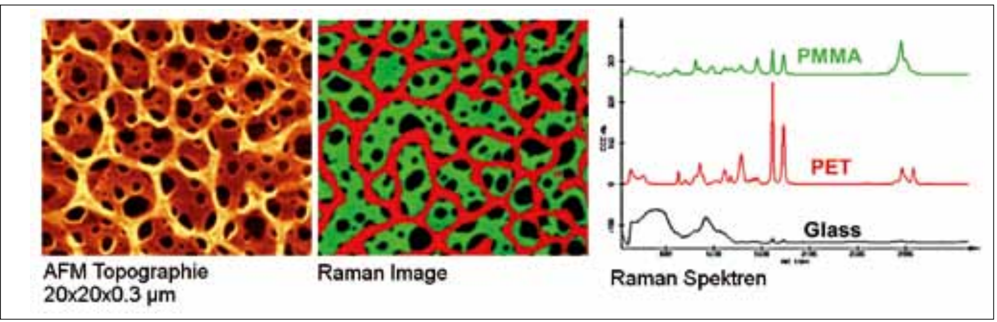

Fig. 5
Topology of a PEET-PET polymer film on a glass substrate recorded with AFM (l.), the Raman spectrum (r.) and the false color representation of the Raman image (center)
(Source: WITec/PI Physik Instrumente)

faster the positioning in the X and Y axis can be. Consequently measurement times are shorter and temperature drift, which would increase with time, is reduced. So the high dynamics also benefit the accuracy.

For these reasons, WITec decided on a piezo-based scanning stage (Fig. 6) from PI (Physik Instrumente) for positioning. It is designed for working distances of 100 or 200 µm in the axes of the scanning plane and 30 µm in the Z axis. It allows a position resolution of better than 2 nm and provides the best conditions for use in modular microscopes for all three methods. As there are no classical mechanical components that could cause friction or mechanical backlash in the drive, very high motion resolution is possible with the piezo drives.

Capacitive sensors and digital electronics ensure stability

Stability and path accuracy during scanning are crucial when combining Raman imaging with AFM, since the measurements can take a few minutes and any drift would distort the recordings. In addition, the active guiding using capacitive sensors increases path accuracy: The sensors measure any deviation in the axis lateral to the direction of motion. Undesired crosstalk of the motion (for example from external forces or mechanical crosstalk) into another axis can be detected in this way and actively compensated in real time.

A digital controller provides the necessary control. It is specially adapted to the piezo-based scanning stage and guarantees a good linearity, also for dynamic operation. The digital electronics work with a high clock rate. This is decisive for an accurate assignment of the position values of the piezo scanner and the recording camera. If it were too slow or inaccurate, there would be a loss of resolution and distortions (jitter) during the assignment. The piezo-based scanning system is an essential part in high-precision microscopes. As a result of its compact dimensions, the scanning stage can be easily integrated in microscopes where installation space is usually limited.

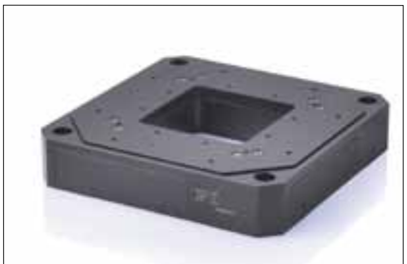

Fig. 6
The piezo-based scanning stage is essential for sample positioning. It is designed for working distances of 100 or 200 µm in the axes of the scanning plane and 30 µm in the Z axis.
It allows a position resolution of better than 2 nm. Capacitive sensors and digital electronics provide maximum stability.
(Source: WITec/PI Physik Instrumente)

Fig. 7
Dipl.-Physiker Gernot Hamann is the Business Development Manager for Microscopy at PI
(Source: WITec/PI Physik Instrumente)