MOVING THE SAMPLE PROPERLY

Markus Wiederspahn explains what to consider when selecting sample stages and lens scanners

Whenever we speak of microscopy, high-performance objectives, sophisticated illumination systems and methods, and sample preparation take centre stage. Modules for moving samples and objectives are important for good working results. Just like you can't get a single horsepower on the road without tyres, microscope users cannot get conclusive images without the precise movement of samples or objectives. Before choosing a sample stage or objective scanner, it is worthwhile analysing the requirements of the planned experiments.

The following questions are crucial. In which and in how many axes is the sample moved? What travel ranges are required? For focusing in the Z direction, what gets moved, the sample or the lens? How accurate does the positioning need to be for the respective motion axes? Can these requirements be fulfilled by a single system or is a combination of stages for rough and fine positioning necessary?

The dimensions of the sample stages, the noise caused by the drive, or the availability of brackets for the inserts are further aspects to consider.

FIG. 2. **The U-780 XY stage from PI, which is suitable for inverse microscopes of different manufacturers, is based on piezo motor technology**

WIDEFIELD MICROSCOPY

The analysis of tissue samples is one of the most common applications for widefield microscopes. The lateral resolution of the microscope is limited by diffraction in the order of magnitude of ≥ 250nm. To be able to repeatedly reach interesting sample areas at high speed, safely, and without aliasing, the bidirectional repeatability of the sample stage has to be at least better by a factor 2.1 than the resolution, that is, it has to be around about 100nm. This derives from the Nyquist sampling theorem borrowed from communications engineering. Piezo ultrasonic stages are able to fulfill all of these requirements.

Z positioning in widefield microscopes is ideally done by means of an objective scanner since often large travel ranges of 100-400 µm and more are required, with a bidirectional repeatability of 200 nm. Short settling times of 20ms help to maintain an optimal workflow. Piezo-based objective positioners even have resolutions of up to 5nm.

FLUORESCENCE MICROSCOPY

Fluorescence microscopy describes numerous microscopy techniques including superresolution. Each technique has its own requirements for sample movement. An example is TIRFM (Total Internal Reflection Fluorescence Microscopy). In this case, the laser beam used for excitation does not hit the sample to be examined orthogonally, but at a flat angle. Therefore, only the molecules close to the surface are excited to fluorescence, which allows an improved resolution in Z direction (100-200nm compared to about 500nm achieved with other microscopy techniques). In order to be able to use this resolution, a correspondingly precise positioning of the sample in the Z direction is required. For this purpose, inserts for the microscope stage that not only offer a lateral positioning accuracy of less than 100nm, but also one in the Z direction are ideal. In this way, the full potential of this technology can be exploited. The GATTAscope, an open-source project from GATTAquant for TIRF microscopy, is an example for this technology.

LIGHT SHEET MICROSCOPY

Lightsheet microscopy (LSFM = Lightsheet Fluorescence Microscopy, also known as SPIM = Single Plane Illumination Microscopy) is also a variant of fluorescence microscopy. Here, the illumination and observation beam paths are divided between two optics arranged orthogonally to each other. The laser beam used to illuminate the sample is focused in only one direction and, therefore, forms an optical section in a plane, the so-called light sheet which illuminates a thin layer of the sample. The fluorescent light that is emitted in this plane is captured and detected by an objective lens. Compared to confocal fluorescence microscopy, this method affects the sample much less. Therefore, LSFM is predestined for the examination of living organisms. One of the research goals of this technology is to gain a better understanding of highly dynamic processes such as embryonic morphogenesis (i.e., the development of organisms, organs and organelles). For this purpose, Z stacks are generated with a thickness of several hundred µm at frame rates of >100 images per second. Depending on the setup of the microscope, either the objective is moved or the sample is moved up and down by means of linear stage. This method is used by the systems created by Professor Jan Huisken and his team at the Morgridge Institute for Research (Madison, WI) for the Flamingo Project. In these systems, the samples are also rotated. For this purpose, rotation stages using ultrasonic piezo motors are employed. Here, speed and uniform motion are decisive. The light sheet Isoview project from Philip J. Keller at HHMI is another example of how the LSFM technology is used.

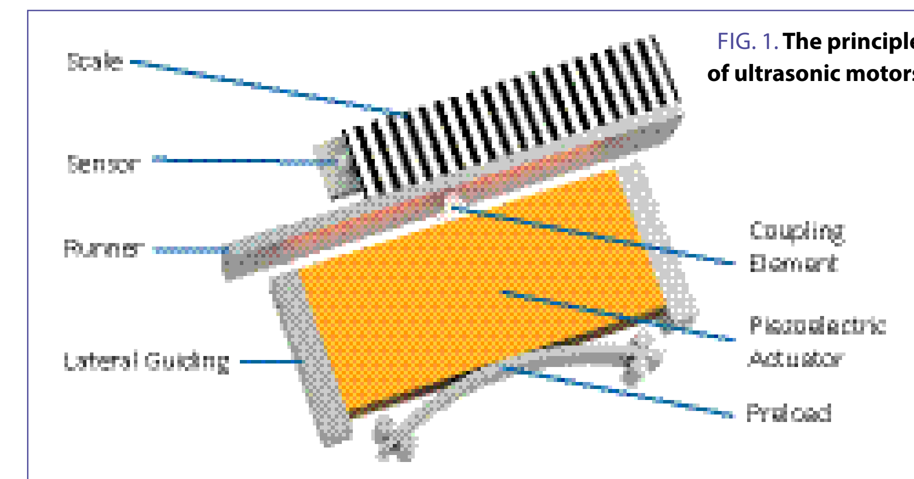

FIG. 1. **The principle of ultrasonic motors**

PIEZO MOTOR XY-STAGES OFFER MANY ADVANTAGES

A variety of drive systems exist for moving samples or objectives – next to motor-drive screw combinations and linear motors, ultrasonic piezo motors are often used. The operating principle is very simple: A piezo actuator is preloaded against a moveable runner by means of a coupling element. High-frequency AC voltage causes the actuator to vibrate at up to 200kHz. The deformation of the actuator leads to a periodic motion of the coupling element relative to the runner. The feed created is a few nanometres per cycle; the high frequencies lead to the high velocities. Even large travel ranges can be easily achieved with this principle.

This principle combines several advantages: The motors are very flat, which is indispensable for inverted microscopes with limited space, so that the sample remains in the focus area of the microscope. In addition, ultrasonic motors are virtually noiseless – this is of great importance in laboratories with several microscopes. Ultrasonic stages also allow very uniform, jerk-free movement, both during slow movements and when approaching defined sample locations at high speeds. If you switch these stages off, they keep their position since they are self-locking. In addition, piezo motors are highly reliable and have a long lifetime.

Piezo technology can also be used directly. The expansion of the piezo crystal, caused by applying electrical voltage, enables high-precision movements with resolutions in the subnanometer range, highest-resolution travel ranges of up to 300 µm and even of up to 2mm with lever amplification.

OTHER REQUIREMENTS

It is also crucial that all required inserts – from the holders for Petri dishes, well plates, and slides up to application-specific inserts – mechanically fit the microscope stage. Last but not least, the software connection plays a significant role. Compatibility with operating software for microscopes and the most important programming languages should be ensured.

CONCLUSION

To be able to make the optimum choice from the wide range of sample stages and objective scanners for microscopy, the constraints of the task must be clarified in detail in advance. Here, it is worth investing time and effort to achieve good results. ●

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