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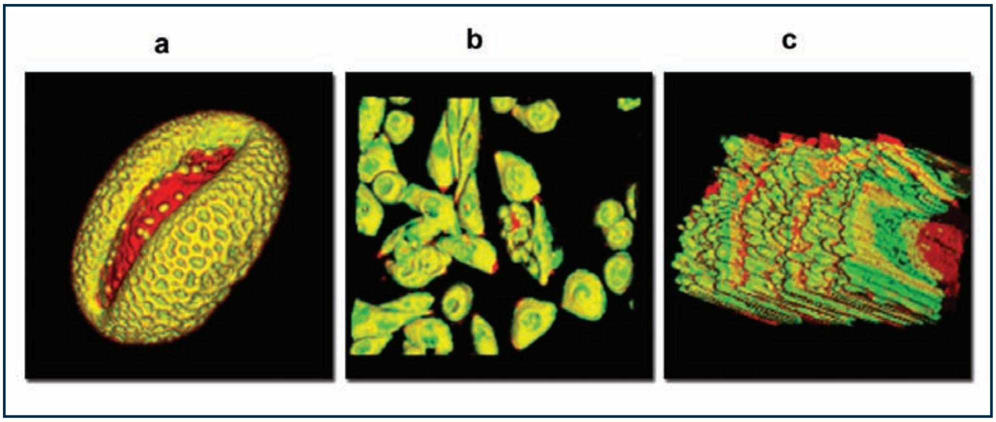
Stage Positioning Provides High-Speed Precision for 3-D Images

by Dr. William O'Brien, Mad City Labs Inc.



Three-dimensional images like these of a pollen grain (a), cells labeled with GFP (b) and mouse intestine require Z-positioning. Accomplishing this with a positioning stage can be faster than doing so by moving the objective. Courtesy of Molecular Expressions.

To acquire three-dimensional information from biological structures requires moving the focus of the microscope vertically along the sample's Z-axis. Gaining this information quickly and accurately is important for time-resolved or video-rate imaging or if the sample may photobleach or be damaged by a long exposure to excitation light. High-speed motion and nanometer precision in the Z-axis are necessary for these types of studies.

Although various options are available for rapid imaging of the X- and Y-planes, Z-axis data collection has typically involved vertically moving the objective lens, and it can take as long as 20 ms per step to achieve nanometer precision. For applications where this is too time-consuming, Z-axis information can be obtained by moving the sample with a nanopositioning stage.

In general, moving the objective lens is difficult because of its relatively large mass and the limited amount of useful space available on most microscopes. Dealing with these limitations places severe design constraints on the objective lens translator. For instance, designers usually want to make a fast-moving component very stiff to maintain precision. However, with microscopes, the objective lens is usually on a nose cone, and space constraints may limit the amount of stiffness that can be added to a translator design.

Using a positioning stage to move only the sample allows the use of stiffer, faster translators to boost positioning speed. Microscopes also tend to have more space available for a stage. Z-positioning requires only one stage per microscope no matter what objective is used, but moving the objective lenses typically requires a positioner for each one used. Thus, a system using Z-positioning costs less and is simpler in design; for instance, there are fewer cables linked to the controller.

A common stage for this type of positioning would include a guidance mechanism, an actuator and a position sensor. For example, a stage could use flexure hinges for guidance. Flexure hinges are unique in that they contain no contacting parts that move relative to each other. The stage moves when strain is placed on the hinge, and its movement is 100 percent reversible, in most cases.

Stage options

In contrast, bearing-guided stages have moving contact positions between the bearings and a hardened surface. The translation of the stage is not reversible at the nanometer level. Piezoelectric actuators, which change length under an applied voltage, supply the driving force, have high load capacities, fast response time and subnanometer resolution.[1] Feedback control is accomplished with an integrated piezoresistive position sensor, which is usually necessary to provide the nanometer-level resolution required of higher-precision nanopositioning.[2] This type of stage exhibits a fast response time, often in the range of 3 ms for a step, with subnanometer resolution.

The typical Z-axis device also includes a large aperture (2.6 × 2.6 in.) with a low profile (0.8 in.). The large aperture is useful for accessing both sides of the sample. An objective lens can be brought up to the sample through the bottom of the aperture, while the top surface of the sample remains accessible to other probes. The low profile is necessary for experiments where it is important to keep changes in the optical path length to a minimum. An example might be an experiment involving phase-sensitivity measurement.

Although stages are available with response times approaching 1 ms, end users usually give up some range of motion in exchange for the higher speed. Typical range of motion, for instance, might be 25 µm. Stages can be built with a range as great as 500 µm, but they do have a slower response time. □

References

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2. W. O'Brien (July 2003). Piezoresistive sensors facilitate high-speed nanopositioning. *PHOTONICS SPECTRA*, pp. 90-92.

Meet the author

William O'Brien is a member of the R&D group at Mad City Labs Inc. in Madison, Wis.; e-mail: obrien@madcitylabs.com.

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