3D Image Acquisition

Closed Loop Drive Technology for Highest Z-Stack Precision

3D Image Acquisition: In fluorescence microscopy, various types of microscopes and digital imaging methods are increasingly used to image samples in three dimensions at highest possible resolution. This is technically achieved by layering images, which are taken at a fixed sample location but at different heights on top of each other.

Improvements in Depth Resolution

Due to the optical transfer function (OTF), each image is overlaid with optical information of its neighbourhood. In axial direction the OTF decreases only slowly leading to an axial resolution remarkably lower than the lateral resolution. Therefore, specialised microscopic techniques and imaging methods focus on improvements in depth resolution. These can be achieved by using special instrumentation and/or mathematical algorithms.

Iterative algorithms improve z-resolution by applying mathematical deconvolution. The calculations require a stack of images taken at axially equidistant positions (z-stack). The possible resolution improvement decisively depends on the precision of the z-adjustment.

Nowadays, precise sample z-movement is realised using piezo or stepper motor technology. These are integrated into the microscope or retrofitted to stages, focus dials or nose pieces (table 1).

The smallest driving increments of motorised system microscopes are stated between 10 and 80 nm. Repeatabilities of piezo shifters range from 10 to 30 nm. Respective specifications for retrofitted z drives are not available. However, an increasing number of microscopes are nowadays retro-fitted with motorised focus components. So the question occurs, if such microscopes are suitable for z-stack acquisition.

Requirements of a 'Z-stack' Acquisition

Examples of theoretical lateral resolution for high-aperture optics are given in row
A corresponding z-resolution requires a z positioning accuracy close to the reciprocal lateral optical resolution.

Z-axis retrofit devices connect to the fine or coarse gear of the focus drive. They use the same high-quality drive components that are also used inside system microscopes. A retro-fitted stepper motor (200 fullsteps/rev), which is driven with 256 micro-steps, achieves 51,200 rotational increments per revolution, leading to a theoretical height resolution of 19 nm at a pitch of 1 mm/revolution. However, mechanical tolerances inside manual focussing drives impose practical limits far below theoretical values.

Gear wheels, the rack pinion gear and sometimes the built in slip rigid coupling lead to real accuracies above 1 µm. Such drives are inappropriate for acquiring high-precision z-image stacks due to the involved mechanics. A proven method of dealing with mechanical inaccuracies is closed loop drive technology. But due to the large variety of geometries and the lack of space for integration this methodology has not been applied to microscopes yet.

The newly developed 'M-Stack' z drive is the first closed loop focus drive, which easily fits to existing manual microscopes. Precision-wise it has been designed to meet the high requirements of a 'z-stack' acquisition. The main components are the drive motor, the linear scale and the position sensor. The key point to integrateability is the extremely space-saving magneto-resistive scale. It is fixed to the microscope's stage support, while the sensor is mounted to the body of the microscope. The motor may connect to either the fine drive or coarse drive of the microscope.
Constant Distance of Images

The position errors slight slope for the M-Stack drive is most probably due to a non-orthogonality between sensor and scale and linearity errors of the linear scale itself. Compared to the internal drive the error is smaller and more linear. The sinusoidal behaviour of the blue error graph can be attributed to the nonlinearity of the stepper motor itself. These errors stem from smallest variations of magnetic gaps inside the motor when rotating from step to step. So in terms of linearity the retrofitted closed loop drive is at least competitive to existing internal drives.

When acquiring z-stacks for subsequent deconvolution the constant distance of images is also extremely important. The respective differential linearity graphs are given in diagram 2. As can be seen, both drive concepts do show practically the same differential error, when moving 100 nm distances repeatedly.

Besides errors due to the drives themselves also thermal expansion and vibrations of the optical construction itself add to the total error. A microscope's thermal expansion behaviour is based on its geometry and the materials used for its components. Assuming, that it is possible to keep the microscope's temperature drift within 0.5 K during a full 'z-stack' acquisition, the respective thermally induced position error is in the range of 150 nm and thus much higher than the error imposed by the M-Stack device itself.

Summary

The newly developed drive with closed loop control eliminates inaccuracies of the internal z-mechanics of manual microscopes reliably.

The achieved absolute accuracy has been measured to be even superior to sophisticated internal microscope drives using ball screw mechanics and micro-stepping motors.

The differential positioning errors range at 25 nm (1 Sigma) for both tested drive concepts. However, a part of the positioning error is due to the accuracy limit of 10 nm of the interferometer itself. The devices differential accuracy is presumably better than presented here.

Authors

Nathalie A. Friedrich, Märzhäuser Wetzlar GmbH & Co. KG, Germany
Contact

Märzhäuser Wetzlar GmbH & Co. KG
In der Murch 15
35579 Wetzlar
Germany
Phone: +49 6441 91160