TN382: Elongation of spherical structures in z

Topic

Fluorescently labelled beads are used for a variety of purposes in optical microscopy, from measuring a point spread function to assessing the performance and calibration of the system. The beads supplied by manufacturers such as Molecular Probes are manufactured under strict controls and can be considered as spheres. This technical note discusses why 3D visualisations of these beads, reconstructed from 2D sections, appear, not as spheres, but as ovoid?

Discussion

The reasons why beads might appear as ellipses instead of spheres in optical microscopy are different depending on the size of the objects being observed.

The z (axial) resolution of a widefield or confocal microscope is normally three to four times less than its xy (lateral) resolution. In other words, the smallest object that can be resolved is approximately 200nm in xy and 700nm in z.

When working with small objects, e.g. 200nm point source beads for measuring a psf, this effect is significant. The beads will appear to be three to four times elongated in z. The elongation effect caused by the difference in resolution in z is only significant on small structures around the resolution limit of the microscope. It is a fact of optical microscopy and should be taken into consideration when interpreting data.

When working with larger structures (>10um) you can sometimes also see elongation. The cause of this is normally different.

In the case of larger structures elongation is due to mismatching of refractive indices, either between the immersion medium of the objective and the sample medium or between the sample medium and the sample itself.

If the refractive indices are mismatched then the physical distance moved by the focus drive is not the same as the optical distance. This gets worse as you move deeper into the sample, away from the coverslip. Optical distance is less than focus drive distance and the result is stretching in z.

To minimise this effect it is recommended that refractive indices are matched as closely as possible. For example if the specimen consists mainly of water and is mounted in an aqueous medium, it would be preferable to use a water immersion lens of a slightly lower NA than an oil immersion lens of the same magnification and higher NA.

Another factor which can also affect elongation is spherical aberration from high NA lenses. Consider evaluating lenses for spherical aberration before accepting the final

example. Spherical aberration is corrected for by proper cover correction on dry or water immersion objectives, using appropriate immersion media to eliminate the refractive index mismatch described above and by using a higher corrected type of lens which corrects for spherical aberration at more colors.

These factors apply to structures within biological samples equally as to beads therefore they should be taken into account when interpreting 3-dimensional data.