

Matching refractive index of mounting media improves axial resolution and image quality in 3D biological samples

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ABSTRACT

Axial resolution in light microscopy can be improved by matching the refractive index (RI) between the biological specimen and the microscope objective. The path of a photon emitted by a fluorophore in a biological sample passes through: 1) the mounting medium in which the sample is embedded; 2) a glass coverslip; 3) immersion oil; and finally 4) the microscope objective. While the optical path between the objective and coverslip typically has a matched RI of 1.52, the majority of biological specimens are mounted in media of substantially lower RI, ranging from 1.42 to 1.47, causing a loss in axial resolution due to refractive index mismatch. Here we use laser scanning confocal microscopy to demonstrate the deleterious effects of mismatched refractive index in conventional mounting medium by measuring the point spread function (PSF) of subdiffraction-sized fluorescent microspheres. We then compare these results with specimens prepared in Invitrogen™ ProLong™ Glass Antifade Mountant, which features an optimized refractive index of 1.52. Our data indicate that at depths beyond 20 μm, an RI-mismatched mounting medium suffers significant losses in axial resolution, whereas the RI-matched mountant maintains optimal axial resolution throughout, enabling up to 75% improvement in axial resolution at focal depths of 100 μm and beyond. By eliminating unwanted refraction, ProLong Glass Antifade Mountant also maximizes the light reaching the objective lens, thus providing higher sensitivity and a brighter image when imaging thick biological samples, such as spheroid cultures that mimic tumor microenvironments. Combined with protection from photobleaching in the visible and near-infrared spectra, the optimized refractive index of ProLong Glass Antifade Mountant measurably increases fidelity and image quality for bright, high-resolution 3D analysis of any cell or tissue type.

RESULTS

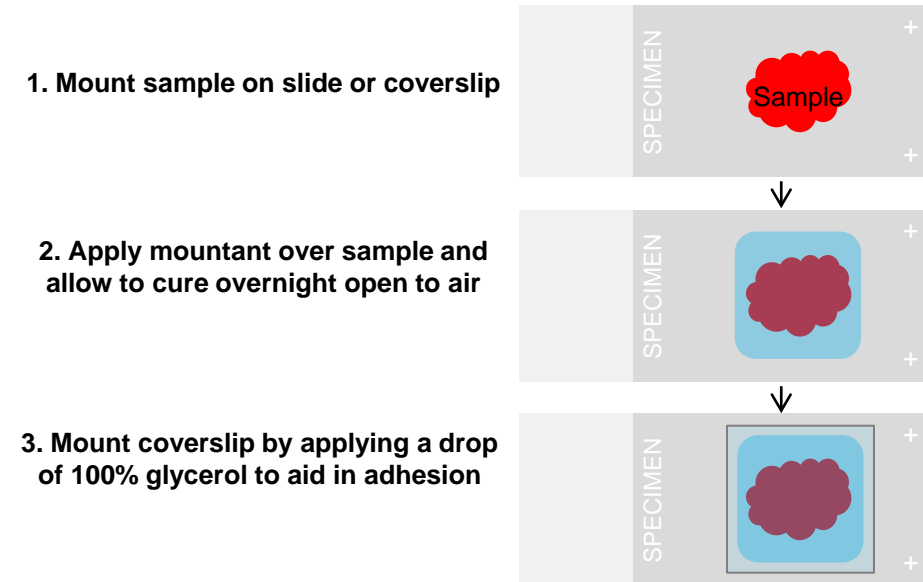
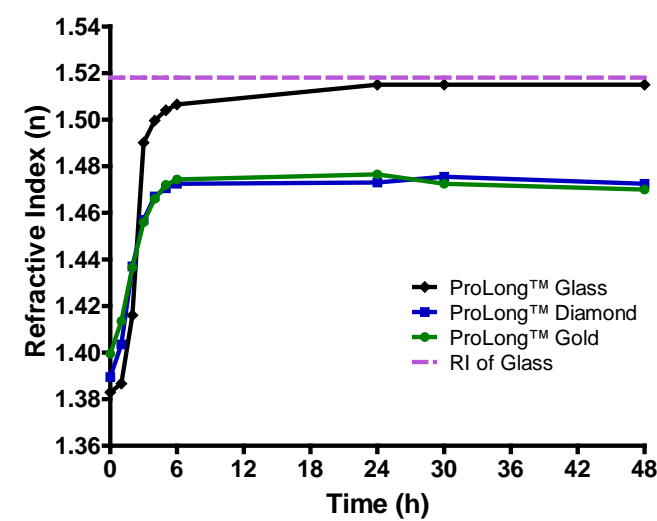


Figure 1. ProLong Glass Antifade Mountant fully cures to a final RI of ~1.52 after 24 hours.

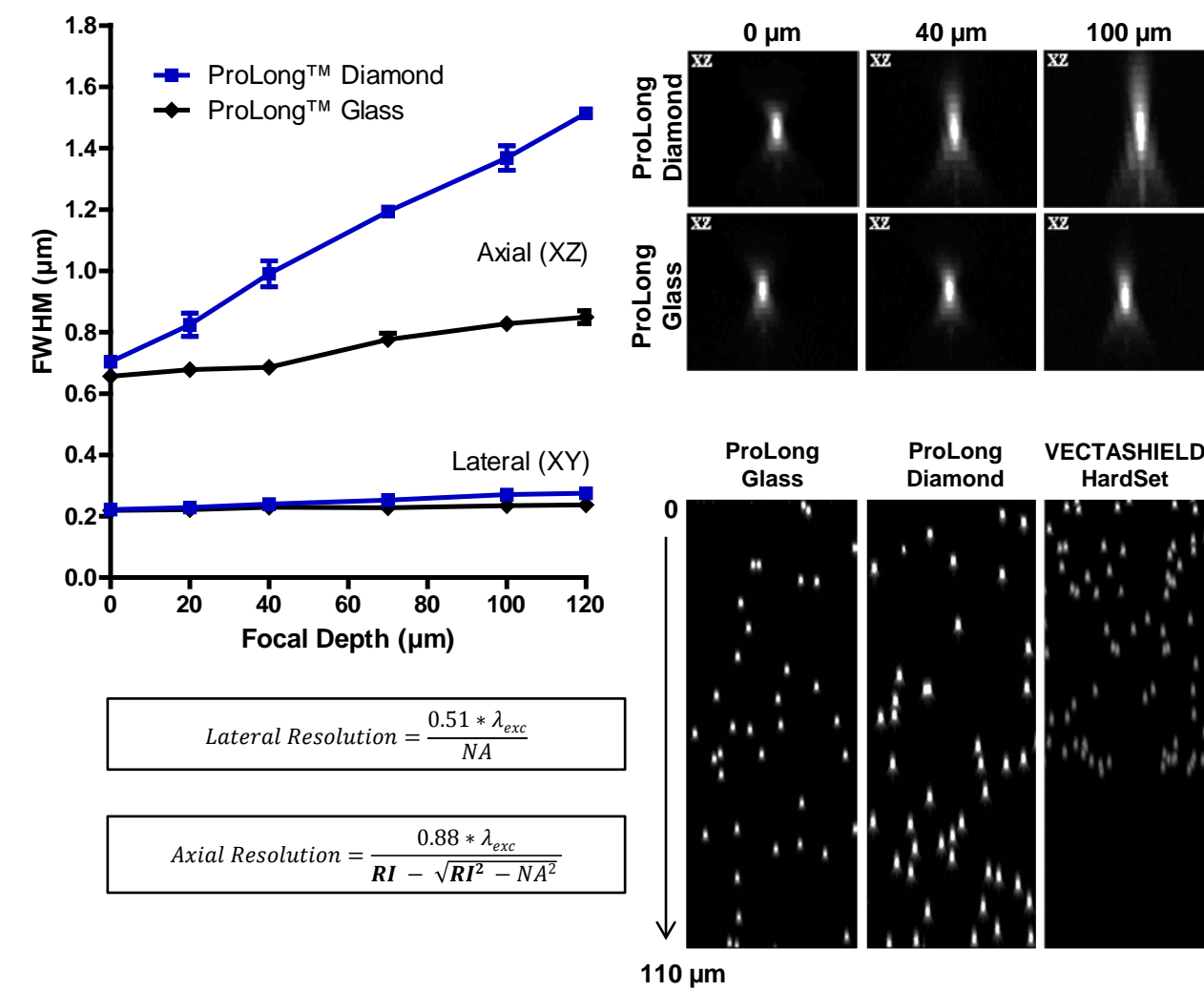


Figure 2. ProLong Glass Antifade Mountant provides superior axial resolution due to refractive index matching.

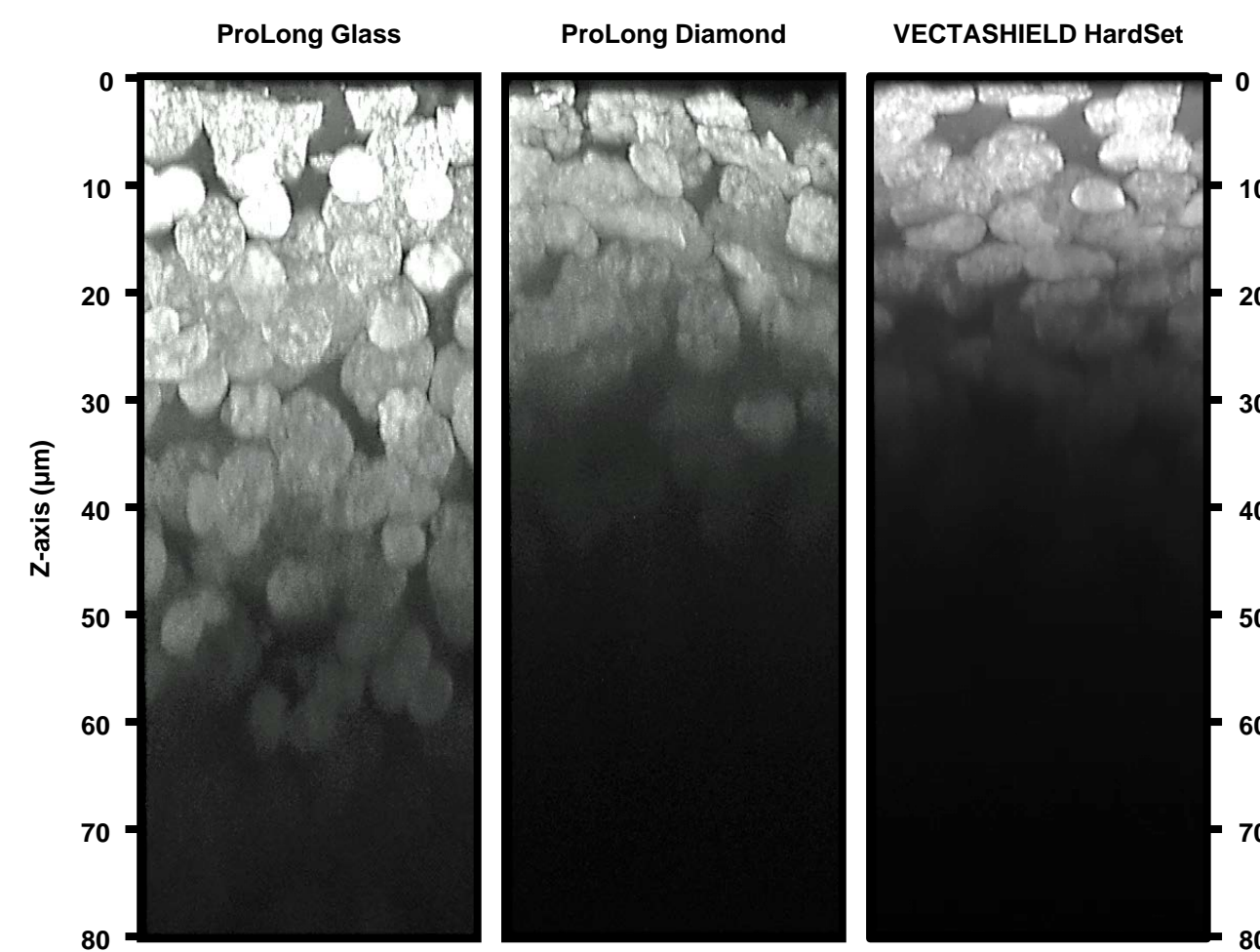


Figure 3. RI matching of ProLong Glass Antifade Mountant allows for deep tissue imaging without noticeable sample shrinkage.

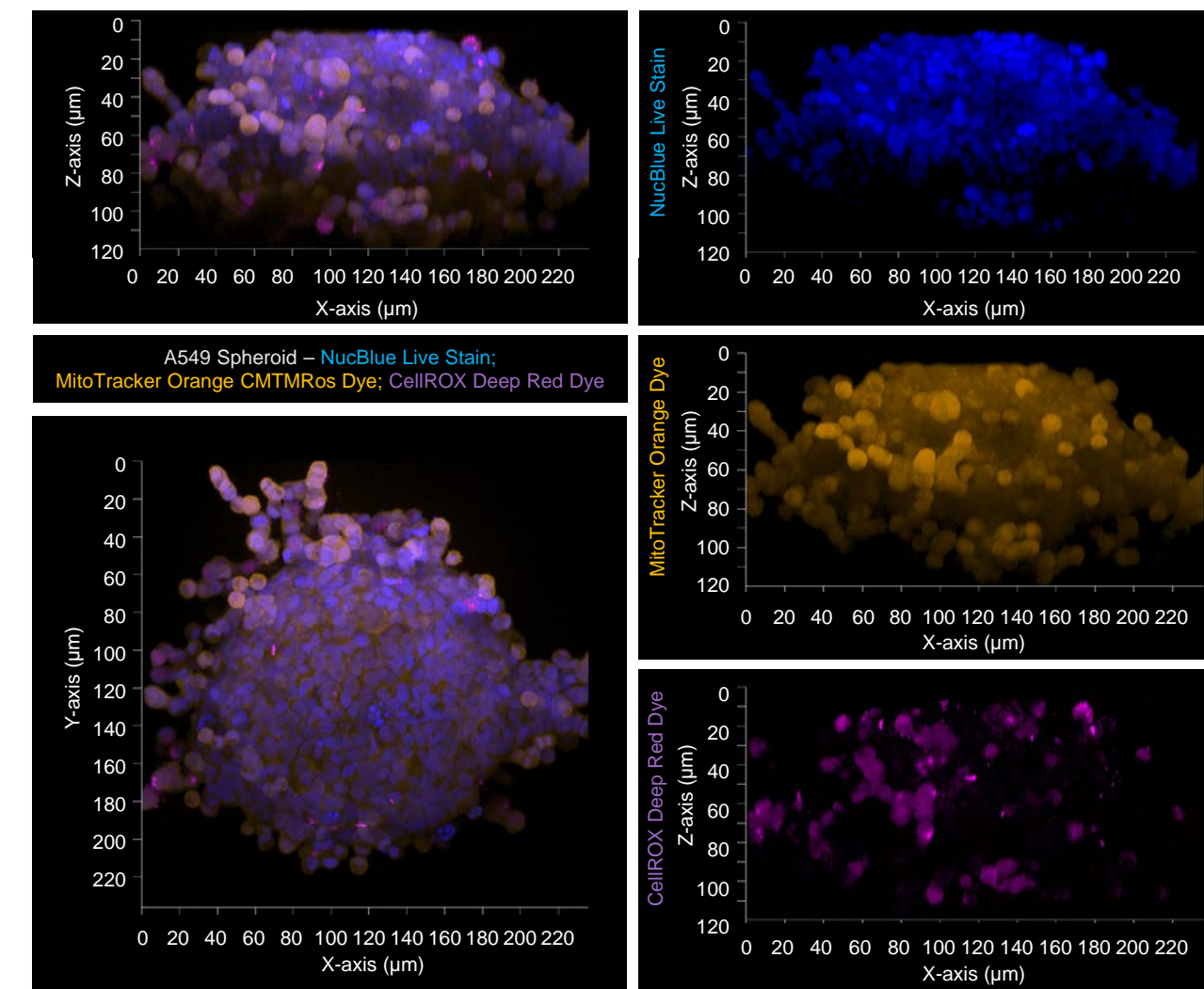


Figure 4. ProLong Glass Antifade Mountant enables high-sensitivity imaging up to 100 μm depths in 3D cultured spheroids.

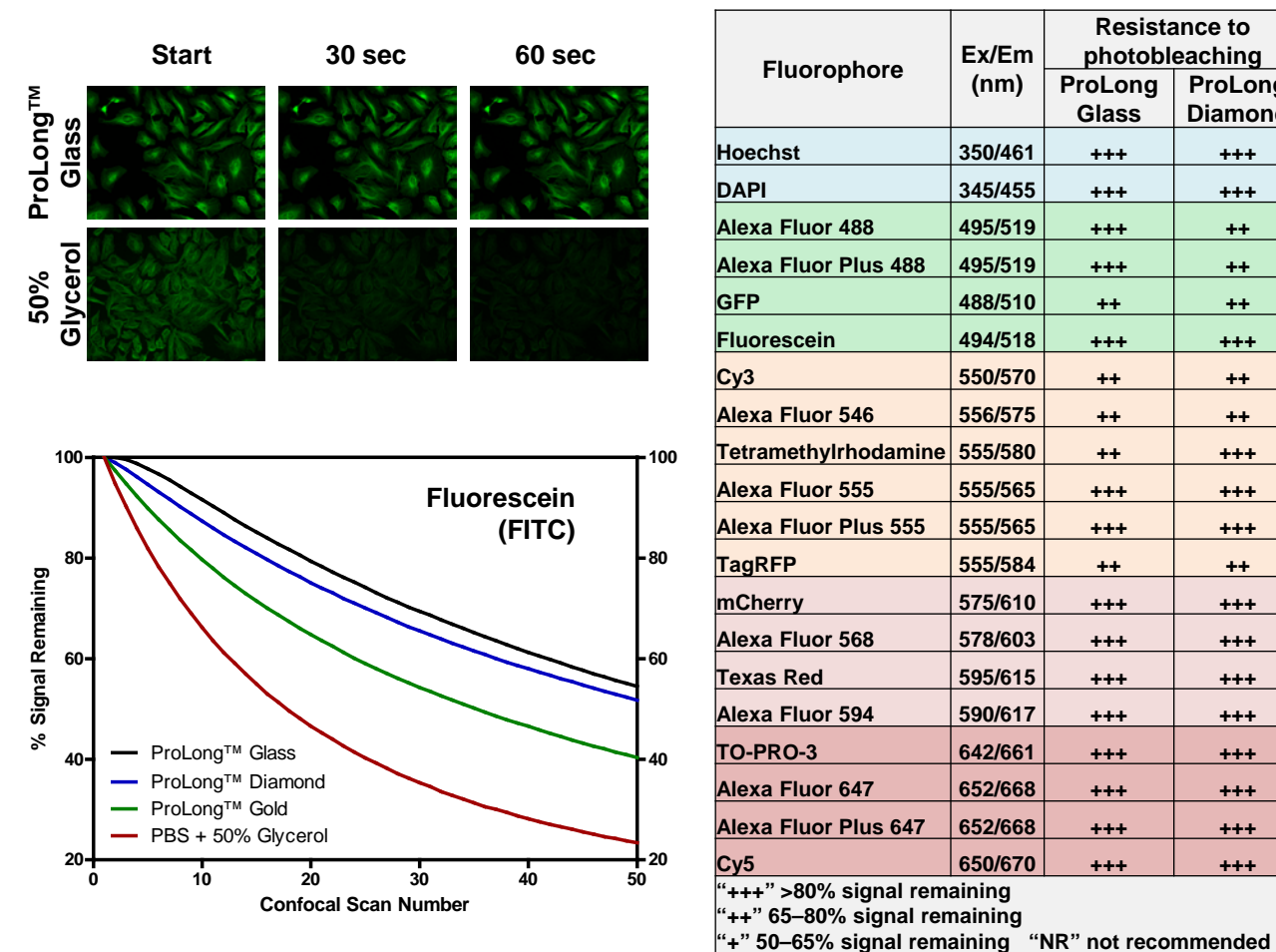


Figure 5. ProLong Glass Antifade Mountant offers superior photobleaching resistance of dyes covering the visible and IR spectrum.

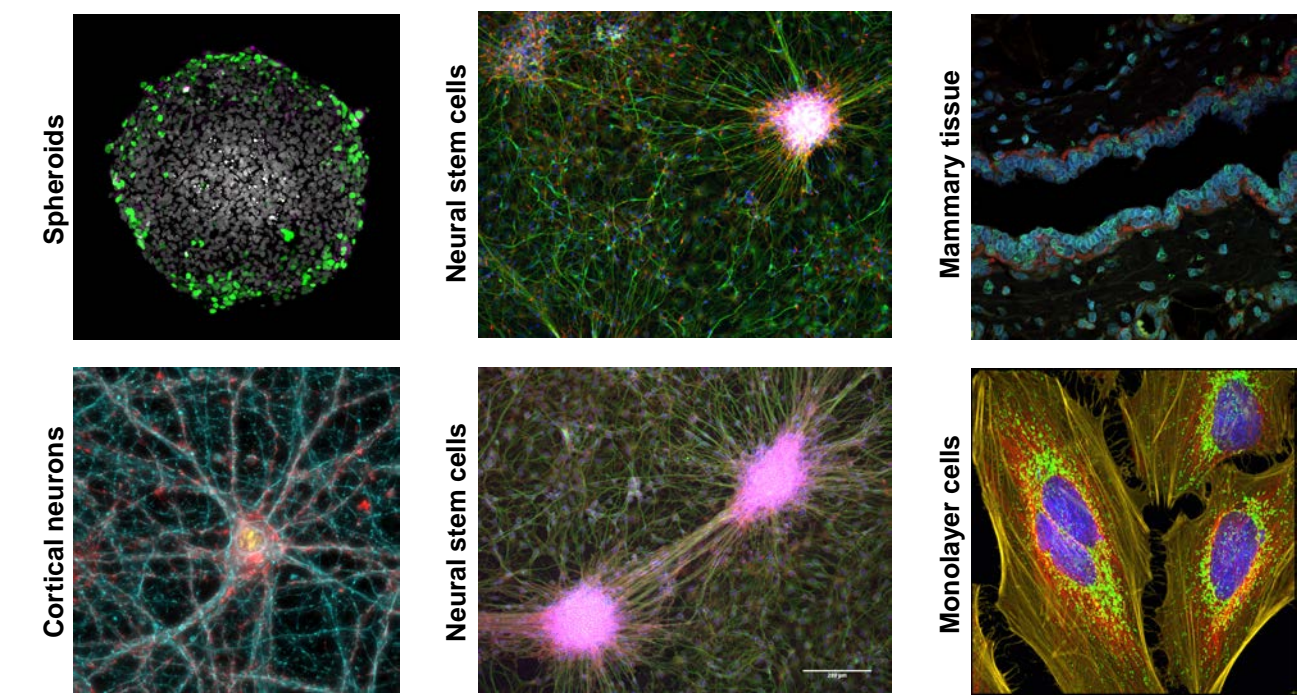


Figure 6. ProLong Glass Antifade Mountant can be used on a wide variety of biological samples.

CONCLUSIONS

ProLong Glass Antifade Mountant is a hard-setting, curing mountant that forms an optical path with minimal distortion. This is achieved by curing to a final refractive index of ~1.52, thus matching the refractive index of the coverslip and immersion oil. When compared to a traditional mounting medium with a mismatched refractive index of ~1.47, the axial resolution on ProLong Glass Antifade Mountant is improved by up to 75% at focal depths beyond 100 μm. Matching the refractive index also improves the light-gathering potential of the objective, thus providing higher signal intensity and improved sensitivity for detection deep within tissues. Combined with its superior performance in protection from photobleaching, these properties make ProLong Glass Antifade Mountant the superior mountant for fluorescence microscopy applications on monolayer cells as well as tissues ranging up to 100 μm in thickness.

REFERENCES

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