Improving the depth resolution of confocal microscopy with low-numerical-aperture objectives using unsharp masking

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The conventional method to improve the depth resolution in confocal microscopy is using high-numerical-aperture (NA) objectives. However, when observing a sample of a large volume, such as an embryo or a multicellular spheroid, the working distance of a high-NA objective usually limits the observation depth to less than 200 μm. In addition, using a high-NA objective means that more scanning steps are required to cover a large field of view. Although a low-NA objective seems to be suitable for observing a sample of a large volume, the depth resolution of a low-NA objective is usually too low to produce reasonably thin optical sections. This is because the lateral resolution of confocal microscopy is proportional to NA^{-1}, while the depth resolution is proportional to NA^{-2} [1]. Therefore, improving the depth resolution of low-NA objectives would be necessary for observing a sample of a large volume.

In this present work we proposed to use the concept of unsharp masking to improve the depth resolution of low-NA objectives in confocal microscopy. Unsharp masking is an old but still popular technique to enhance the fine features in images [2]. Instead of using a numerically blurred original image as the mask, here we captured two axially offset images (one above and the other below the in-focus confocal image by a depth of focus) with the pinhole fully opened as the masks. Then we chose a suitable intensity of the two masks (40–80% of the in-focus confocal image) and subtract them from the confocal image. The results in the figure below shows that this method could improve the depth resolution of a confocal image obtained with a NA 0.7 objective by 39%. More details will be introduced in the conference.

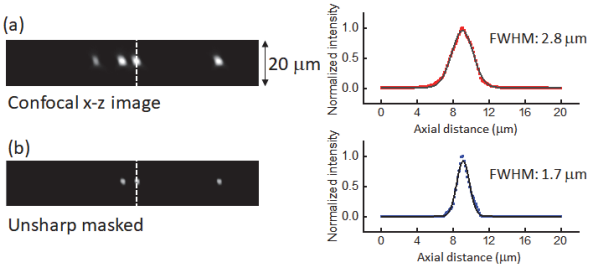

Figure. (a) Confocal x-z image of four 1-μm fluorescent beads (emission peak: 550 nm) captured by a NA 0.7 objective. The curve shows the intensity profile along the dashed line. (b) Image and intensity profile of the image in (a) after unsharp masking. The width of the intensity line profile is reduced by 39%.

REFERENCE

1. D. B. Murphy and M. W. Davidson, *Fundamentals of Light Microscopy and Electronic Imaging*, 2nd ed. (John Wiley & Sons, Hoboken, New Jersey, 2013), Chap. 13.
2. *Ibid*, Chap. 18.