Supplementary information of "Axial Plane Optical Microscopy"

Tongcang Li[1,2,†], Sadao Ota[1,†], Jeongmin Kim[1], Zi Jing Wong[1], Yuan Wang[1, 2], Xiaobo Yin[1, 2], and Xiang Zhang[1, 2, *]

[1]NSF Nano-scale Science and Engineering Center, 3112 Etcheverry Hall, University of California, Berkeley, California 94720, USA.
[2]Material Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720, USA.

[†]These authors contributed equally to this work

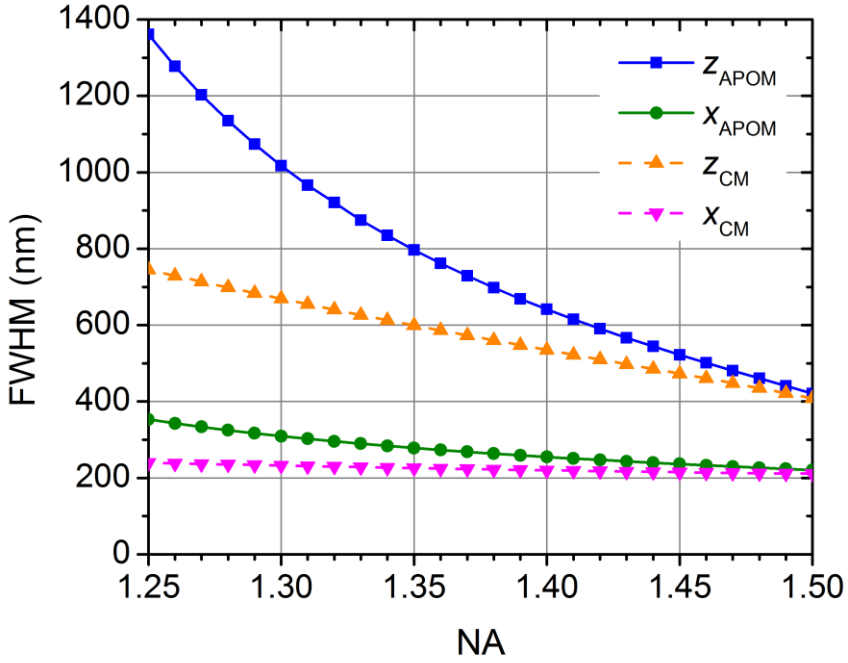
*Corresponding author. E-mail: xiang@berkeley.edu

Supplementary Figure 1	Calculated FWHM of point spread functions
Supplementary Figure 2	A SEM image of the calibration nano-hole array
Supplementary Figure 3	Imaging a fluorescent bead behind a 75 μm-thick brain slice

Note: Supplementary Videos 1, 2 are available on the Scientific Reports website.

Supplementary Figure 1

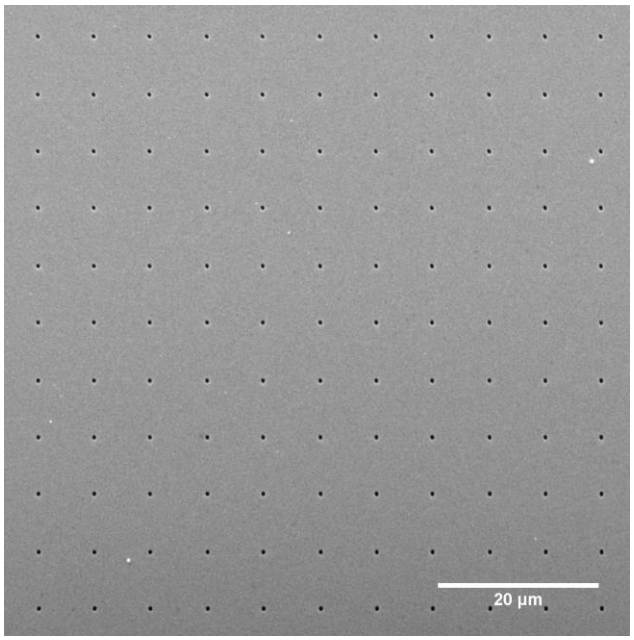
Calculated FWHM of point spread functions



Calculated FWHM of the PSF of APOM and a conventional wide-field microscope (CM) along the *x* (lateral) and *z* (axial) axes as a function of the NA of objective lenses for the wavelength of 546 nm. Note that a CM cannot image the axial plane directly. We calculate the FWHM of the PSF of a CM for comparison only. The PSF is not symmetric along the *x* and *z* axes for both APOM and CM's. The resolution of APOM approaches that of CM for high NA objective lenses.

Supplementary Figure 2

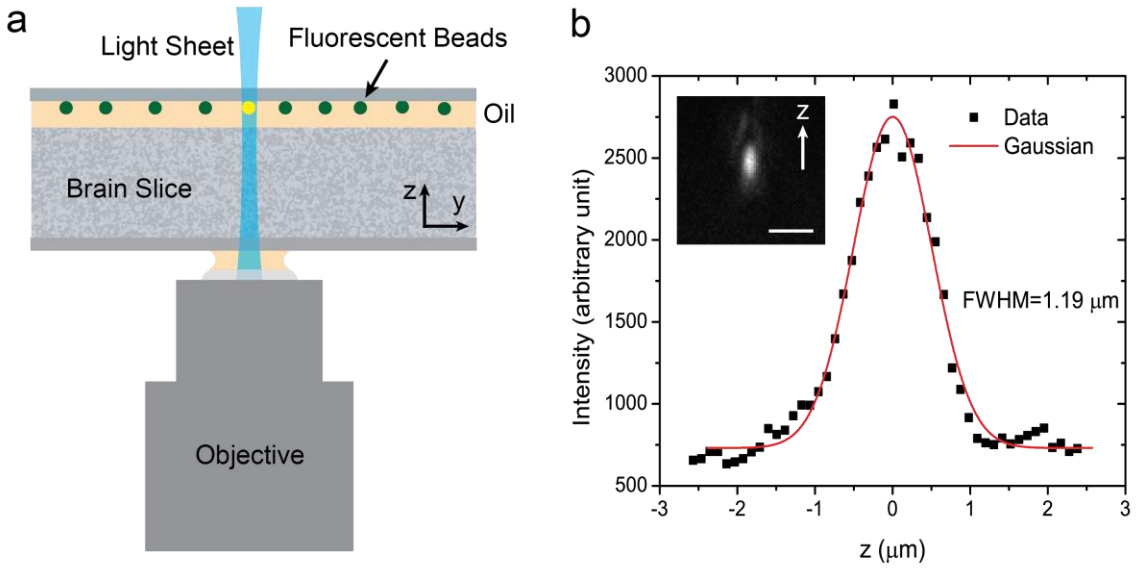
A SEM image of the calibration nano-hole array



A SEM image of the nano-hole array used for calibration of the APOM. The period of the array is 7 μm, and the diameter of each hole is 500 nm. The nano-hole array is fabricated by a focused ion beam (FIB) on a 150 nm gold film deposited on a glass coverslip. The small defects (bright spots) near the right top corner and the left bottom corner of the nano-hole array are also visible in the optical image taken by APOM (Fig. 2d in the main text).

Supplementary Figure 3

Imaging a fluorescent bead behind a 75 μm-thick brain slice



Because we used oil immersion objective lenses in this experiment, the resolution of APOM may degrade due to the mismatch in the refractive index of biological samples and the immersion oil. To estimate the performance of APOM for biological applications, we have imaged 200 nm diameter green fluorescent beads placed on top of a 75 μm-thick brain slice (a). The fluorescent beads were stuck on the surface of a glass cover slip. We put a thin layer of immersion oil between the cover slip and the brain slice to make sure that there was no air gap between the beads and the brain slice, and match the refractive index. The fluorescent signal passed through the immersion oil and the 75 μm thick brain slice before it was collected by the objective lens (NA=1.4). The measured FWHM of the bead is about 1.19 μm in the axial direction (b). This shows that we can obtain micrometer-resolution images of thick biological samples with APOM, which is also demonstrated by Fig. 3 and Fig. 4 in the main text. The inset of (b) is an image of a fluorescent bead. Scale bar: 2 μm.