



Supplementary Figure 1. Axial and lateral resolution depends on depth in brain slices. **a**, Bead (4.7 μm) fluorescence decreases with depth within the molecular layer of a cerebellar brain slice. Peak fluorescence was defined as the peak of a Gaussian fitted to a 3D image of a bead at a given depth. Total focal brightness was defined as the peak fluorescence times the width of the 3D Gaussian fit. **b**, Lateral full-width half-maximum (FWHM) resolution at various depths for 1 μm and 4.7 μm bead fluorescence and uncaging of MNI-glutamate on dendritic branchlets of CA1 pyramidal cells. **c**, Average voltage-clamp responses from MNI-glutamate uncaging on CA1 pyramidal cells on a shallow (8 μm from surface) dendrite and a deep (40 μm) dendrite. Response amplitudes decrease as the uncaging volume is moved downward. **d**, Axial FWHM resolution at various depths for 1 μm and 4.7 μm beads and for double-caged (bis-CNB) and single-caged (MNI) glutamate.