Abstract:
In vivo Ca2+ imaging of neuronal populations in deep cortical layers has remained a major challenge, as the recording depth of two-photon microscopy is limited because of the scattering and absorption of photons in brain tissue. A possible strategy to increase the imaging depth is the use of red-shifted fluorescent dyes, as scattering of photons is reduced at long wavelengths. Here, we tested the red-shifted fluorescent Ca2+ indicator Cal-590 for deep tissue experiments in the mouse cortex in vivo. In experiments involving bulk loading of neurons with the acetoxymethyl (AM) ester version of Cal-590, combined two-photon imaging and cell-attached recordings revealed that, despite the relatively low affinity of Cal-590 for Ca2+ (Kd=561 nM), single-action potential-evoked Ca2+ transients were discernable in most neurons with a good signal-to-noise ratio. Action potential-dependent Ca2+ transients were recorded in neurons of all six layers of the cortex at depths of up to -900 µm below the pial surface. We demonstrate that Cal-590 is also suited for multicolor functional imaging experiments in combination with other Ca2+ indicators. Ca2+ transients in the dendrites of an individual Oregon green 1,2-bis(o-aminophenoxy)ethane -N,N,N',N'-tetraacetic acid-1 (OGB-1)-labeled neuron and the surrounding population of Cal-590-labeled cells were recorded simultaneously on two spectrally separated detection channels. We
conclude that the red-shifted Ca2+ indicator Cal-590 is well suited for in vivo two-photon Ca2+ imaging experiments in all layers of mouse cortex. In combination with spectrally different Ca2+ indicators, such as OGB-1, Cal-590 can be readily used for simultaneous multicolor functional imaging experiments.