A fundamental challenge in neuroscience is the determination of the three-dimensional (3D) morphology of neurons in the cortex. Here we describe a semiautomated method to trace single biocytin-filled neurons using a transmitted light brightfield microscope. The method includes 3D tracing of dendritic trees and axonal arbors from image stacks of serial 100-microm-thick tangential brain sections. Key functionalities include mosaic scanning and optical sectioning, high-resolution image restoration, and fast, parallel computing for neuron tracing. The mosaic technique compensates for the limited field of view at high magnification, allowing the acquisition of high-resolution image stacks on a scale of millimeters. The image restoration by deconvolution is based on experimentally verified assumptions about the optical system. Restoration yields a significant improvement of signal-to-noise ratio and resolution of neuronal structures in the image stack. Application of local threshold and thinning filters result in a 3D graph representation of dendrites and axons in a section. The reconstructed branches are then manually edited and aligned. Branches from adjacent sections are spliced, resulting in a complete 3D reconstruction of a neuron. A comparison with 3D reconstructions from manually traced neurons shows that the semiautomated system is a fast and reliable alternative to the manual tracing systems currently...