Fluorescence signals from in vivo two-photon imaging conditions are often weak, resulting in a poor signal quality. The acquisition of such fluorescence signals from small structures like dendritic spines, the site of synapses in brain neurons, are especially difficult due to their small size, making temporal observations of physiological parameters like changes in ion concentrations challenging. The signal quality can be increased by reducing the excitation power and image size, and increasing the image frequency [1]. To avoid the scanning of signal-free tissue space around the neurons, we developed a scanning procedure for the acquisition of fluorescence data selectively from the objects of interest, namely the obliquely oriented dendrites of neurons of cortical regions in vivo in animal models of brain function.

Here, we present a method for the acquisition of 2D images with trapezoid-geometry and 3D orientations at variable angles. The acquisition of such 2D raster images is important for an understanding of signal integration in single neurons in the living brain. For the implementation of this method, we used a scanning device consisting of an acousto-optical deflector, a galvanic mirror and an electrical tunable lens [2] for the X,Y, and depth-position of the focus spot, respectively. This arrangement allows the acquisition of images at frame rates of 40 Hz, and the detection of synaptic function-related calcium signals in spines on dendrites with oblique orientations throughout a dendritic length of at least 50 microns in cortical neurons in the intact brain.