The past two decades saw the emergence of two-photon laser-scanning microscopy as a powerful imaging tool for thick tissue imaging. Owing to its inherent optical sectioning capability, deep penetration of the excitation light in tissues, and its capability to excite multiple fluorophores, two-photon laser-scanning microscopy has been used in a wide range of imaging applications. The minor drawback, however, of this imaging technique is its relatively slow image acquisition rate. In this study, we demonstrate a novel and simple scanless two-photon imaging technique based on the generation of an illumination plane. We applied this novel technique to depth-resolved imaging of cameleon-expressing pharyngeal muscle of C. Elegans. This technique offers high acquisition rates, minimal photodamage, and deep penetration of excitation light into thick tissues allowing long-term dynamic imaging of wide range of biological samples from embryos to adult worms.

Figure 1: Fluorescence optical sections of cameleon in C. elegans pharyngeal muscle at 8 µm depth intervals clearly depicting the optical sectioning capability of two photon imaging based on plane illumination. Scale bar: 50 µm.