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Abstract: We use an ultrafast diode-pumped semiconductor disk laser (SDL) to demonstrate several applications in multiphoton microscopy. The ultrafast SDL is based on an optically pumped Vertical External Cavity Surface Emitting Laser (VECSEL) passively mode-locked with a semiconductor saturable absorber mirror (SESAM) and generates 170-fs pulses at a center wavelength of 1027 nm with a repetition rate of 1.63 GHz. We demonstrate the suitability of this laser for structural and functional multiphoton in vivo imaging in both Drosophila larvae and mice for a variety of fluorophores (including mKate2, tdTomato, Texas Red, OGB-1, and R-CaMP1.07) and for endogenous second-harmonic generation in muscle cell sarcomeres. We can demonstrate equivalent signal levels compared to a standard 80-MHz Ti:Sapphire laser when we increase the average power by a factor of 4.5 as predicted by theory. In addition, we compare the bleaching properties of both laser systems in fixed Drosophila larvae and find similar bleaching kinetics despite the large difference in pulse repetition rates. Our results highlight the great potential of ultrafast diode-pumped SDLs for creating a cost-efficient and compact alternative light source compared to standard Ti:Sapphire lasers for multiphoton imaging.

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