EFFICIENT NONLINEAR EXCITATION OF ENCODED FLUORESCENT PROTEINS IN LIVING SAMPLES USING A SEMICONDUCTOR DISK LASER

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1. ABSTRACT

To fully exploit the potential of nonlinear (NL) microscopy and enable its integration in clinical studies, inexpensive, simple, compact, efficient and sample-compatible ultrafast laser systems must be designed. Based on this, sample damage and NL signal enhancement criteria [1]; we propose the use of a compact (140x240x70 mm) semiconductor disk laser (SDL) to efficiently produce two-photon excited fluorescence (TPEF) and second harmonic generation (SHG) in living *C. elegans* samples. The SDL is modelocked by a quantum-dot semiconductor saturable absorber mirror (SESAM), enabling it to deliver 1.5 ps pulses at 500 MHz. The output average power of this source is 287 mW. This high repetition rate enables to balance the tradeoff between the desired high average intensity and unwanted NL photodamage as the obtainable NL emission is limited by the strong peak intensity and high pulse energy of the laser [2]. Besides this, its wavelength is centered at 965 nm, ideally suited for TPEF of the widely used Green Fluorescent Protein (GFP) as it virtually matches its two-photon action cross section [3]. Under these conditions, a few tens of milliwatts of excitation power are enough to excite this protein thus maximizing sample viability. Importantly this compact SDL could be used as a platform to develop portable nonlinear bio-imaging for clinical studies.

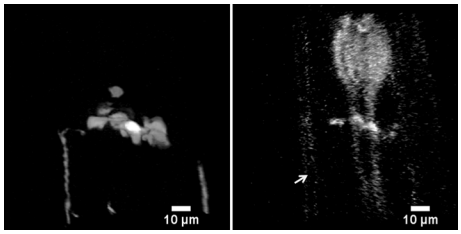

Figure 1: Left panel is the TPEF signal from GFP expressing motoneurons and nerve-ring of a *C. elegans* nematode. Right panel SHG signal from pharynx and body wall muscles (see arrow).

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