

WIDE-FIELD SECOND HARMONIC GENERATION MICROSCOPY OF BIOLOGICAL TISSUES IMAGED USING DIFFERENT LASER PULSE REPETITION RATES

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Ultra-fast, high power lasers are required to obtain efficient second harmonic generation (SHG) contrast in a wide-field microscope. However, to avoid photodamage, biological tissues and live samples cannot be exposed to very high pulse peak powers. Therefore, a careful selection of laser parameters, such as pulse repetition rate, energy per pulse as well as imaging area, are required to optimize non-linear wide-field microscopy imaging. In this work, we report on a wide-field SHG microscope, suitable for fast imaging of live samples and large area imaging of histology sections.

We use a high repetition rate amplified laser system (PHAROS®, Light Conversion Inc) providing variable pulse repetition rate up to 1 MHz and compare with imaging with a home built SESAM mode-locked Yb:KGW oscillator providing 60 MHz pulse repetition rate and up to 12W of average power. The photobleaching effects are found to be different depending on the laser parameters as well as on the spectral properties of imaged biological samples. The

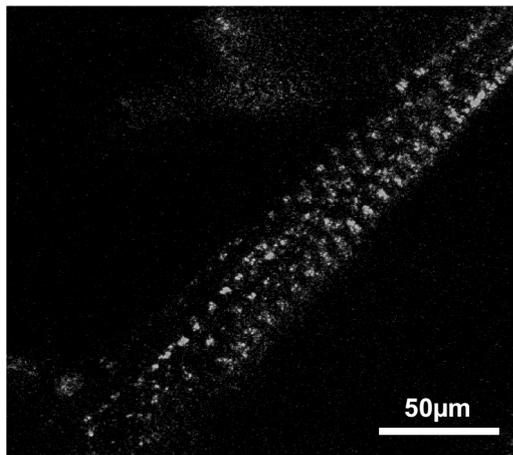


Figure 1: SHG imaging of fruit larva muscle (100ms integration time).

imaging of histology sections and live recording of moving *Drosophila melanogaster* larvae is presented. It is demonstrated that high spatial resolution wide-field microscopy is able to distinguish between the anisotropic A-bands and the isotropic I-bands, present in the characteristic striated muscle structure (Fig. 1). SHG intensity flickering that correlates with sarcomere length is shown. The wide-field live imaging of contracting *Drosophila* larvae is achieved with a laser power of 4W at the sample over a 400x400 μm area using 60 MHz pulse repetition rate Yb:KGW oscillator. The work shows that wide-field non-linear microscopy technique provides excellent temporal resolution for large imaging area.

[1] V. E. Kisel, A. S. Rudenkov, A. A. Pavlyuk, A. A. Kovalyov, V. V. Preobrazhenskii, M. A. Putyato, N. N. Rubtsova, B. R. Semyagin, and N. V. Kuleshov, "High-power, efficient, semiconductor saturable absorber mode-locked Yb:KGW bulk laser," *Opt. Lett.* **40**, 2707-2710 (2015).

[2] D. Tokarz, R. Cisek, A. Golaraei, S. Asa, V. Barzda and B. C. Wilson, "Ultrastructural features of collagen in thyroid carcinoma tissue observed by polarization second harmonic generation microscopy", *Biom. Opt. Exp.* **6**, 3475-3481 (2015).