

# WIDEFIELD NONLINEAR MICROSCOPE FOR REAL TIME IN-VIVO ANALYSIS

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Nonlinear optical microscopy has emerged as a successful tool within the bio-medical research field enabling the possibility to perform imaging of intact living organisms. The exploitation of nonlinear optical effects (NLO) requires high optical peak intensities that are usually achieved by focusing pulsed lasers through a microscope objective, thereby decreasing the field of view dramatically and obliging to employ scanning solutions to increase the imaged area to values comparable to the widefield illumination. As result, the acquisition time increases to several seconds, preventing the study of time resolved dynamics over a large area in the millisecond regime, where a number of physiological processes occur. In this work, we report on a new setup to overcome these limitations, suitable for widefield real time nonlinear microscopy imaging in living organisms, where a precise selection of laser parameters and imaging setup is required to optimise the final images. In this respect a femtosecond laser (Pharos, Light Conversion) acts as primary light source for an OPA (Orpheus F, Light Conversion) which is coupled to a confocal laser scanning microscope (FV3000, Olympus), enabling the unique possibility to control the NLO effect by means of repetition rate (1 - 50 KHz), pulse duration (50 – 170 fs) and wavelength (600 - 2600 nm). With this new approach images with a field of view up to about 1 mm<sup>2</sup> can be obtained, recording dynamical processes with frame rates up to 100 fps in the complete

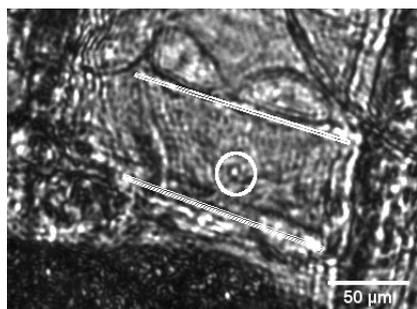


Figure 1: Zoom of *Drosophila melanogaster* heart larval (white lines) containing a NP (white circle).

area. This new setup is suitable to do fast nanoparticle tracking in mesoscaled organisms. In this respect, harmonic nanoparticles are chosen as biological markers because of their high nonlinear efficiency [1], absence of blinking and good biocompatibility. This way, dynamic processes inside the heart chamber of *Drosophila melanogaster* are studied, demonstrating that the entire heart can be imaged, keeping a high spatial resolution to track the nanoparticles. This provides experimental access to the velocities of the hemolymph flux in this closed biological. Financial support DFG INST 190/179 FUGB is gratefully acknowledge.

## References

[1] Z. Wang, C. Kijatkin, A. Urban, M. Haase, M. Imlau and K. Koempe, "Nonlinear Optical Potassium Niobate Nanocrystals as Harmonic Markers: The Role of Precursors and Stoichiometry in Hydrothermal Synthesis Nanoscale", *Nanoscale* **10**, 10713-10720 (2018).