

UNSTAINED *DROSOPHILA* EMBRYO DEVELOPMENT ANALYSED BY VELOCIMETRIC THIRD HARMONIC GENERATION MICROSCOPY

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During embryo development, cell movements are highly regulated in time and space. Because *Drosophila melanogaster* serves as a major model in developmental genetics, morphogenetic movements involved in *Drosophila* embryo development are of particular interest and many mutant strains exhibiting altered movements are available. However, due to the highly scattering nature of early stage embryos, direct imaging of *Drosophila* development is difficult. Moreover, fluorescent labelling can introduce unwanted perturbation and might be difficult to obtain in complex mutants. As a result, a complete quantitative description of these complex dynamical processes is still lacking in many cases.

THG microscopy was recently proposed as a novel technique for obtaining structural images of biological samples with micrometer 3D resolution. By associating this technique with particle image velocimetry (PIV) algorithms adapted from hydrodynamics, we have demonstrated micron-scale quantitative measurements of tissue velocity fields inside unstained opaque embryos [1,2]. Optical properties of *Drosophila* embryos were characterized using a combined 2PEF/THG home-built microscope. We showed that sustained THG imaging does not perturb embryo development by following sensitive developmental dynamics and comparing the survival rate of embryos with and without imaging. Finally, we illustrated velocimetric THG imaging by quantifying morphogenetic movements in live unstained wild-type and mutant embryos.

Our results establish third harmonic generation (THG) microscopy as a novel powerful approach for visualizing and quantifying morphogenetic movements *in vivo* in unstained embryos.

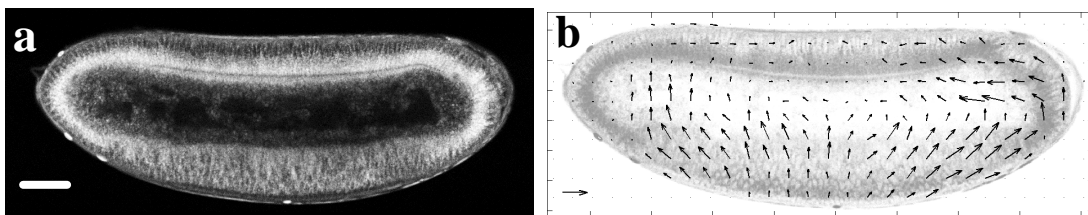


Figure 1: a, unstained *Drosophila* embryo visualized by THG microscopy (scale bar, 50 μ m); b, displacement field obtained by PIV analysis (scale arrow, 5 μ m/min)

References:

- [1] D. Débarre, W. Supatto, E. Farge, B. Moulia, M.-C. Schanne-Klein, and E. Beaurepaire, "Velocimetric third-harmonic generation microscopy: micrometer-scale quantification of morphogenetic movements in unstained embryos", *Opt. Lett.*, **29**, 2881-2884 (2004)
- [2] W. Supatto, D. Débarre, B. Moulia, E. Brouzés, J.-L. Martin, E. Farge, and E. Beaurepaire, "In vivo modulation of morphogenetic movements in *Drosophila* embryos with femtosecond laser pulses", *Proc. Nat. Acad. Sci. USA.*, **102**, 1047-1052 (2005)