

***Ex vivo* multiphoton microscopy using intrinsic signals for pharmacological studies in cardiac and vascular tissue.**

**A.-M. Pena, T. Boulesteix, M.-P. Sauviat, E. Beurepaire, M.-C. Schanne-Klein
Laboratory for Optics and Biosciences CNRS-INSERM, Ecole Polytechnique,
F-91128 Palaiseau, France.**

Keywords: multiphoton microscopy, *ex vivo* 3D imaging, second harmonic generation, sarcomere, myocytes, collagen, elastin, vessel walls

We report novel applications of multiphoton microscopy for pharmacological studies of unstained cardiovascular tissue. We used endogenous sources of nonlinear signals and achieved molecular or structural specificity by combining two-photon-excited fluorescence (2PEF) and second harmonic generation (SHG) microscopy using appropriate spectral filters.

First, we showed that multiphoton microscopy of unstained cardiac myocytes can be used to determine the sarcomere length with sub-resolution accuracy, owing to the remarkable contrast of the SHG signal originating from myosin filaments. A measurement precision of 20 nm is achieved, taking the sample variability into account. We used this technique to measure sarcomere contracture in the presence of saxitoxin, and results were in agreement with mechanical measurements of atrial tissue contracture^[1]. Finally, detailed analysis of images of sarcomere shortening provided strong evidence that the source of intrinsic SHG signal lies in the myosin heads.

Second, we characterized multiphoton microscopy of fresh unlabeled vessels. We performed simultaneous detection of 2PEF from elastin laminae and SHG from collagen fibers upon 860 nm excitation. We showed that combined 2PEF/SHG images provide a highly specific, micron scale description of the architecture of these two major components of the vessel wall. We used this methodology to study the effects of a pesticide on the artery wall structure, and evidenced structural alteration of the vessel morphology.

These functional applications demonstrate that multiphoton microscopy using intrinsic signals is a promising tool in nanopharmacology of unlabeled cardiac and vascular tissue.

[1] T. Boulesteix, E. Beurepaire, M.-P. Sauviat et M. C. Schanne-Klein, "Second harmonic microscopy of unstained living cardiac myocytes: measurements of sarcomere length with 20 nm accuracy", *Optics Lett.* **29**, 2031-2033 (2004)

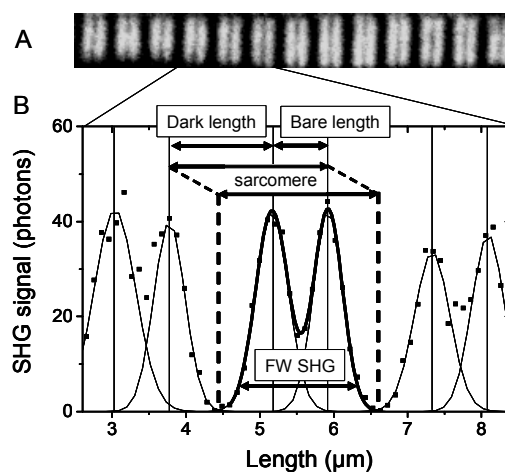


Fig. 1: SHG image (A) and signal profile (B) along a single atrial myocyte isolated from an adult frog heart. The solid lines correspond to fitting with multiple Gaussians.

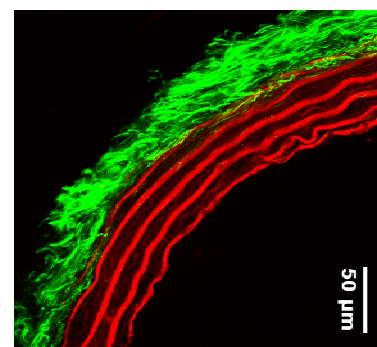


Fig. 2: combined 2PEF/SHG image of a rat unstained carotid artery