UNIVERSITÉ DE GENÈVE
FACULTÉ DES SCIENCES

Geneva, June 24th, 2013

Dear Editor,

Please find enclosed our manuscript entitled *"Harmonic nanoparticles for regenerative research"*.

Since a few years we witness a steady increase in the number of multi-photon imaging platforms in universities, hospitals, and industrial R&D departments. This rising interest for nonlinear imaging is motivated by several factors, both scientific and commercial. The former include the inherent three dimensional sectioning capabilities and high spatial resolution of nonlinear approaches, accompanied by the possibility to work in the infrared spectral region, increasing penetration depth and minimizing sample absorption and degradation. The commercial reasons, on the other hand, comprise the increased availability of cost-effective ultrafast lasers and the fact that, in 2009, the patent on 2-photon microscopy ran expired, favoring the opening of this market to several manufacturers. These facts have motivated ours and a few other research groups worldwide to work on the development of "harmonic nanoparticles", a novel family of *inherently nonlinear* microscopy probes. The optical contrast mechanism they exert, contrary to quantum dots and up-converting nanoparticles, is not based on fluorescence but on second harmonic generation. This physical mechanism enables a series of attracting optical properties including excitation-wavelength tunability, complete absence of bleaching and blinking, and narrow (tunable) emission bands. These characteristics, in turn, make these nanosystems amenable for long-term tracking and deep tissue imaging. A context where these properties might be particularly favorable is that of regenerative medicine. In fact, despite the increasing success of stem cells based approaches, a complete understanding of the integration mechanism of stem cells or derivative into a native tissue after transplantation is still missing. This lack of knowledge descends primarily from the difficulty to track in a continuous fashion and for extended time periods (days to weeks) transplanted cells within tissues at sub-cellular spatial resolution. With our video protocol we show for the first time that harmonic nanoparticles can be used to monitor the evolution of embryonic stem cells, follow their differentiation, and even provide three-dimensional information about the beating pattern of cardiomyocites.

We believe that this contribution is particularly suited for JoVE, as it combines different state-of-the-art approaches (non linear nanoparticles, harmonic microscopy, embryonic stem cells) providing sound results and data analysis.

Staying at your disposal for any further information, I remain

Yours sincerely,

Dr. Luigi Bonacina

Corresponding author: Luigi Bonacina
e-mail: luigi.bonacina@unige.ch
GAP - Biophotonics
Université de Genève
22, chemin de Pinchat
CH-1211 Genève 4

Phone: + 41 22 379 05 08
Fax: + 41 22 379 05 59