Label-free time-gated *in vivo* imaging of *Hydra vulgaris* with luminescent porous silicon nanoparticles as long-lived probes

C. Schiattarella[1,2*], R. Moretta[1], T. Defforge[3], G. Gautier[3], C. Tortiglione[4], B. Della Ventura[2], M. Terracciano[5], L. De Stefano[1], R. Velotta[2], I. Rea[1]

1 Institute for Microelectronics and Microsystems, CNR, Naples, Italy
2 Department of Physics, University of Naples "Federico II", Naples, Italy
3 GREMAN UMR-CNRS 7347, Université de Tours, INSA Centre Val de Loire, Tours, France
4 Institute of Applied Sciences and Intelligent Systems, CNR, Pozzuoli, Italy
5 Department of Pharmacy, University of Naples "Federico II", Naples, Italy

**chiara.schiattarella@na.imm.cnr.it*

Highly luminescent porous silicon nanoparticles are herein functionalized and exploited for high-contrast in vivo time-gated fluorescence imaging employing Hydra vulgaris as target organism.
Keywords: Luminescent nanomaterials; *in vivo* imaging

1. Introduction

Luminescent silicon nanostructures have gained great attention as label-free probes for bioimaging and theranostic applications in the recent years, thanks to the demonstrated biocompatibility, well-established surface chemistry and peculiar optical properties [1]. Herein, we present an imaging study employing surface-modified porous silicon nanoparticles (PSiNPs) as label-free probes. Specifically, *Hydra vulgaris* is used as *in vivo* model for internalization studies by virtue of its tissue-like structural complexity [2]. The peculiarly bright background tissue autofluorescence represents a significant obstacle when carrying out *in vivo* imaging studies. This issue can be bypassed by exploiting the long PL lifetime of PSiNPs, paving the way to the use of the time-gated imaging technique [3].

2. Results and Discussion

Luminescent PSiNPs were fabricated by electrochemical etching of highly resistive p-doped silicon wafer following an established protocol [4] and stored in isopropanol. To prevent the uncontrolled Si-H → Si-O-Si surface bonds substitution in pristine NPs when put in aqueous environment, a coverage of PSiNPs was realized following a two-step process. Firstly, a thermal hydrosilylation with undecylenic acid was performed in order to achieve a covalent capping with Si-C chemistry and simultaneously expose carboxyl functionalities; then, poly-l-lysine (PLL) was conjugated via electrostatic interaction. Hydrosilylated PSiNPs (hPSiNPs) were already characterized and showed a PL quantum yield > 10%, µs-range emission lifetime and morphological stability for up to 24 h observation [5]. The change in sign of the surface charge (- to +) observed after PLL coverage of hPSiNPs (PLL@hPSiNPs) resulted of utmost importance to promote the internalization in *Hydra* [2]. Background tissue autofluorescence represents a common issue in any fluorescence-based imaging technique, since it crucially worsens the probe contrast. Herein, we can efficiently get rid of the ns-lived endogenous component from *Hydra* organism by exploiting the µs-lived PL lifetime of PLL@hPSiNPs, thus temporally discriminating the signals applying a proper time gating (Fig. 1).

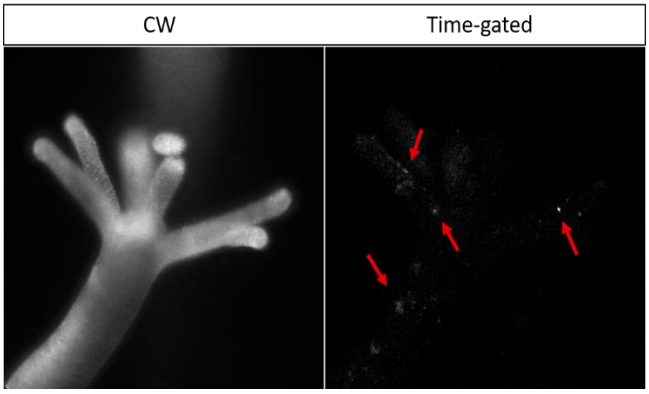

Fig. 1. Continuous-wave and time-gated *in vivo* imaging of *Hydra vulgaris* after 2h incubation with hPSiNPs_PLL.

3. Conclusions

We have presented surface-modified luminescent PSiNPs as label-free probes and their time-gated imaging application. Indeed, we have shown that we can pursue an effective temporal discrimination of the signals and a remarkable improvement of the image contrast with respect to continuous-wave acquisition.

References

1. Park, J.-H. et al., *Nat. Mater.* **8**, 331 (2009).
2. Tortiglione, C. et al., *PLOS ONE* **4**, 11, e7698 (2009).
3. Joo, J. et al., *ACS Nano* **9**, 6233 (2015).
4. Joo, J. et al., *Appl. Phys. Lett.* **108**, 153111 (2016).
5. Schiattarella, C. et al., *Appl. Phys. Lett* **114**, 11, 113701 (2019).