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Quantifying calcium concentration in living cells through detection of photoluminescence single photon trails

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Silicon photomultipliers (SiPM) are solid-state photodetector consisting in arrays of hundreds to thousands of Single Photon Avalanche Diodes (SPADs) per mm^2. They feature a photon detection efficiency in excess of 40% at the peak sensitivity wavelength and guarantee an unprecedented photon number resolution at room temperature. These properties, along with low operation voltage, compactness, and robustness, make SiPMs excellent devices for light detection from single to several thousand of photons, especially when fastest timing is required.
Beyond High Energy and Nuclear Physics, SIPMs are employed in nuclear medical imaging, quantum optics, functional optical spectroscopy and biophysics, where SIPMs are exploited to detect fluorescence and chemiluminescence light.
In this paper, the potential of this class of sensors was exploited in a novel application: the measurement of calcium concentration gradients in living cells. Calcium ion plays a crucial role in several biological processes (e.g., muscle contraction, neurotransmission, cell signal transduction pathways...) and the measurement of spatial and temporal variations of the concentration of this ion is essential to understand, and eventually tune by means of suitable drugs, the underlying mechanisms behind such processes.
A method to measure calcium concentration is based on the quantification of the chemiluminescence generated by aequorin, a calcium-sensitive photoprotein. Upon binding to calcium ions, it generate signals consisting in a sequence of single photons. This method was so far exploited using custom designed systems based on Photo Multiplier Tubes (PMT), limited in portability, flexibility and cost effectiveness, motivating a study based on SiPM. As a proof of concept, a 6x6 mm^2 SiPM-based setup was fully qualified in terms of dynamic range, response linearity, sensitivity, and limit of detection, before being successfully applied in live cell measurements, with a limit of detection greater than what was previously measured with PMTs. Two read out techniques, integrating the produced charge or counting single photons, were compared, showing that the latter approach results into a better sensitivity, even if non-linearity effects due to pile-up were observed at rates beyond a few MHz [1].

The project is now moving forward, and a new multi-sensor setup equipped with a 10x10 mm^2 square matrix of 64 independent 1x1 mm^2 SiPMs is currently under test. This parallelized configuration extends the dynamic range in counting mode by more than 30 times and introduces the possibility of spatial discrimination, paving the way for aequorin-based calcium imaging. Moreover, the new platform incorporates a perfusion system allowing extracellular medium substitution and drug administration, and the whole setup is placed in a compact box to protect the detector from environmental light and to allow measurements on site. In this respect the sensor is operated at stable temperature by using an active cooling system based on Peltier cell. This will also allow us to contain the dark count rate.
Besides the standard instrumental qualification, calibration curves for the absolute measurement of calcium concentration were obtained exploiting the above-described setup, while more complex experiments involving the spatial variable are still ongoing, with the aim of approaching calcium imaging by means of suitable optics.

In future, the feasibility of exploiting SiPMs matrices-based setup to discriminate and give a quantitative estimation of water contaminants triggering cell calcium gradients, as the estrogenic endocrine-disrupting chemicals (EEDCs), will be explored. EEDCs are associated with breast and
prostate cancer, affect reproduction of humans, domestic and wild animals, and the standard techniques (as liquid or gas chromatography combined with mass spectrometry) to measure EDCs are usually expensive and they cannot be run in site. In this respect, a SiPMs based assay could provide a cheaper and on-site pre-screening of the contaminants actually present in a water sample leading to a more sensitive analysis by standard analytical methods only for the detected contaminants.

[1] F. Ruffinatti, S. Lomazzi, L. Nardo, R. Santoro, A. Martemiyanov, M. Dionisi, L. Tapella, A. Genazzani, D. Lim, C. Distasi and M. Caccia, "Assessment of a Silicon-Photomultiplier-Based Platform for the Measurement of Intracellular Calcium Dynamics with Targeted Aequorin,"ACS Sens. 2020, no. 5, pp. 2388-2397, 2020.

In-person participation

Yes

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