NEW FLIM TECHNOLOGIES FOR SINGLE-CELL BIOCHEMICAL SENSING AT HIGH SPATIO-TEMPORAL AND BIOCHEMICAL RESOLUTION

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1. BACKGROUND

Cellular processes, including growth, migration, and death, are regulated through complex and dynamic networks of biomolecular interactions. The understanding of biochemical networks is essential to model and predict cellular decisions. For instance, recent advances from our lab based on a family of multiplexed FRET-based biosensors (NyxBits), permitted us to reveal significant cell-to-cell variability in biochemical networks and cellular phenotypes that underly therapeutic response to anticancer drugs [1]. This work required the specialized in-house development of detection electronics, but a new generation of detectors are becoming commercially available promising to eliminate existing barriers to broad adoption.

2. FAST AND COST-EFFECTIVE FLIM FOR SINGLE-CELL BIOCHEMISTRY

The development of cost-effective, high-speed FLIM technologies have revolutionized the imaging of dynamic cellular events. Initial developments of all-solid-state FLIM systems based on time-of-flight detectors [2] have led to the recent commercial implementations of fast FLIM cameras [3,4] (**Fig 1**). These cameras provide an integrated FLIM system which lowers the barriers to entry for lifetime imaging. We will demonstrate the development of an imaging platform integrating cost-effective pulsed lasers, a phase-modulated 2D solid-state PCO.FLIM camera [5], and a microfluidic system for fast imaging of FRET-based genetically-encoded metabolic biosensors. We will present this development aiming to share an open platform [6] that will make available to the broader community fast single-cell biochemical (metabolic/signalling) with high spatio-temporal and biochemical resolution.

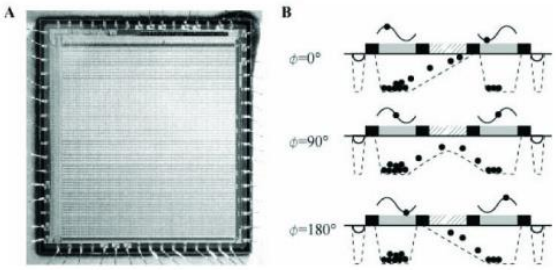

Fig 1: The lock-in imager sensor. **(A)** Microphotography of the sensor. **(B)** Each pixel has two gates that are controlled with voltages in opposite phase. Thus, the photoelectrons generated in the photosensitive area will accumulate in the two storage areas according to the relative phase of the photon flux and gate potential. Figure from [2].

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[2] A. Esposito, *et al.*, *Optics Express*, 2005, 13: p. 9812-9821;
[3] M. Raspe, *et al.*, *Nature Methods*, 2016, 13: p. 501–504;
[4] R. Franke, *et al.*, *Proc. SPIE*, 2015, 9328 93281K;
[5] PCO.FLIM (https://www.pco.de/flim-camera/pcoflim/);
[6] ATLAS.ONE (https://wp.me/p6m2Op-dD)