Innovating solid state detection technologies for biomedical imaging

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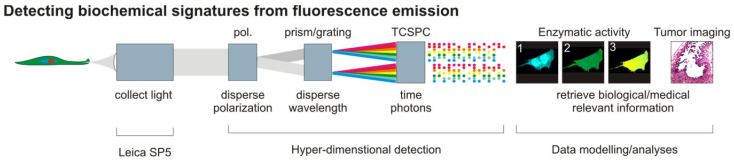
Background

Fluorescence microscopy is an invaluable tool to probe cell and tissue biochemistry. Fluorophores and fluorescent sensors are sensitive to the physico-chemical properties of the environment and thereby encode biologically relevant information into changes of their photophysical properties.

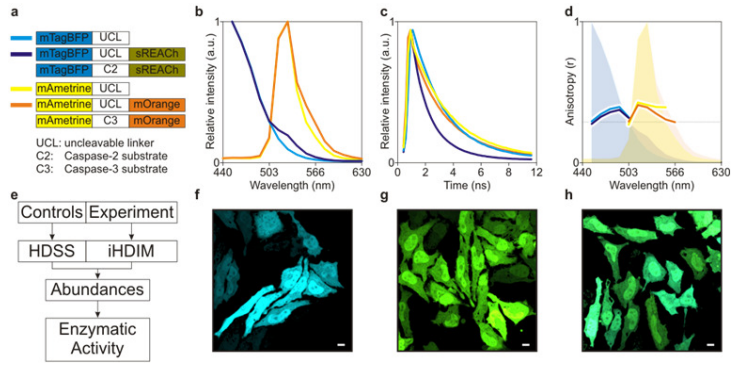
Limited photon-budget hinders the capability of biophysical imaging techniques to reflect small changes in biochemical systems and to un-mix complex biochemical signatures. Hyper-Dimensional Imaging Microscopy (HDIM) enables parallel detection and analysis of all properties of fluorescence (spectra, lifetime and polarization).



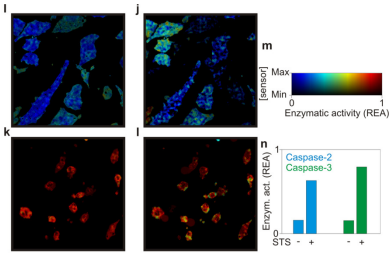
To be applicable in biology/clinic the technology should be cost-effective and user-friendly as well. This is the area where solid state detection can provide a significant impact.

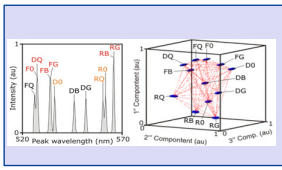
HDIM application: FRET multiplexing

Plasmids coding for Caspapse-2 and Caspase-3 sensors, uncleavable controls and donor-only controls were cloned with a novel combination of fluorescent proteins (mTagBFP/sREACh and mAmetrine/mOrange) (**a**). The two FRET pairs can be both excited at 840 nm and emit fluorescence in different, but overlapping, spectral windows (**b**). The presence of FRET can be detected by changes in fluorescence lifetime (**c**) or fluorescence anisotropy (**d**) for the blue or yellow/orange FRET pairs. In panel (**d**), the emission spectra are shown in the background and the horizontal grey line represents the intensity threshold over which anisotropy is computed. HDIM data analysis (**e**) thus permits to quantify enzymatic activities. The sensors are well expressed as show by true colour representations of HeLa cells expressing the Caspase-2 (**f**), Caspase-3 (**g**) or both biosensors (**h,i-m**).



The relative enzymatic activities of Caspase-2 (**i,k**) and Caspase-3 (**j,l**) measured with non-treated (**i,j**) and Staurosposporine (**k,l**) treated (4 M/6hrs) doubly-transfected HeLa cells are also shown. A net increase of enzymatic activity is apparent (**n**).

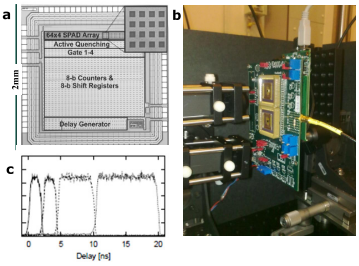




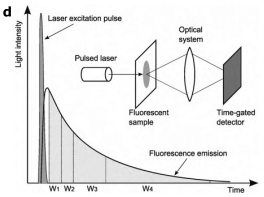
For a generalized strategy to optimize multi-dimensional optical detection and enhance biochemical resolution in microscopy please visit our poster
[P1-C/11] *The photon partitioning theorem: Enhancing biochemical resolution in microscopy*
A. Esposito, M. Popleteeva & A.R. Venkitaraman

Solid state detection

The FluoSPAD detector (**a**) based on single photon avalanche diodes (SPADs) was designed at Foundazione Bruno Kessler (Trento, Italy) to enable fast parallel lifetime sensing in spectrally and polarization resolved applications.

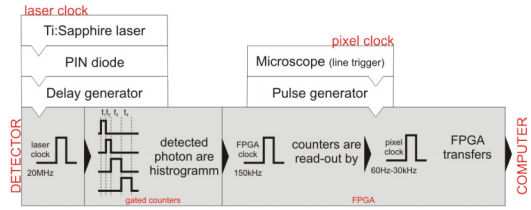


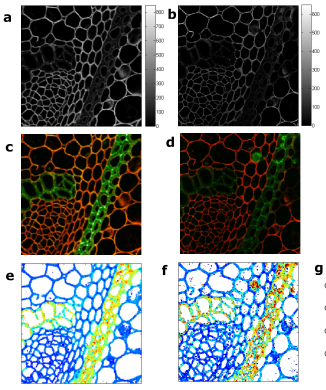
The detector consists of two sensor chips (**b**); each of them incorporates 64 single SPADs operated in parallel. Histograms of photon-arrival times are formed by four sequentially gated counters (**c,d**) enabling in-pixel fluorescence lifetime sensing.



Spectrally resolved FLIM

The system is ran by an FPGA and communicates with a standard PC just via USB, providing spectrally resolved FLIM (soon also HDIM) with a maximum achievable count-rate of 320MHz.



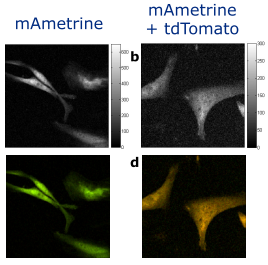


Intensity (**a**), RGB (**c**) and lifetime images (**e**) of *Convallaria majalis* were acquired with this system in 8.5 sec at 20MHz laser repetition rate. To collect the same amount of photons the traditional TCSPC system acquiring at maximum allowed count rate to avoid pulse pile-up and with 80MHz laser repetition rate requires 2 min (**b, d,f**). Histogram of fluorescence life-time as measured by the SPAD- (blue line) and the PMT- (red line) based systems (**g**).

Detection of fluorescent proteins

The current prototype has only 11% sensitivity (at 600nm) and 34% fill factor. Already now acquisition of challenging biological samples begins to be feasible. Improved prototypes are likely to provide significant advantages compared to commercial TCSPC systems.

Intensity (**a,b**) and RGB (**c,d**) images of HeLa cells expressing mAmetrine (**a,b**) and mAmetrine fused to tdTomato.



Conclusion

The parallel detection and analysis of all properties of fluorescence enhances biochemical resolution and enables multiplexing several biochemical reactions at the same time. Furthermore, solid state detectors provide such capability with a cost-effective and user-friendly turn-key system that can pave the way for routine HDIM applications in the biomedical field.

References

Pancheri and Stoppa, ESSCIRC 428–431 (2009)
Esposito *et al*. Opt. Express, 19(3):2546–55 (2011)