A Compact Lifetime Fluorescence Detector for Clinical Diagnosis

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Fluorescence Lifetime Spectroscopy

**FLS (Fluorescence Lifetime Spectroscopy):** a diagnostic technique based on the differences in the exponential decay rate of the fluorescence from a fluorescent sample.

**Pros:**
- Fluorescence lifetime is a property of individual fluorophores.
- Can be combined with measure of the fluorescence intensity.
- The time-resolved bioassays separate the fluorescence of interest from the background through lifetime differences.

**Procedure:**
- Fluorescence Lifetime is obtained by employing the Time-Correlated Single Photon Counting (TCSPC) technique with a Single Photon sensitive detector such as a SPAD.
- The sample is repetitively exited by a pulsed light source, to create the fluorescence emission.
- The time of arrival of the emitted photons is measured and a histogram is built, following the decay function.

The development of Point of Care devices for the early diagnosis of current relevant diseases of our society needs to achieve novel miniature and very effective sensors ▶ ▶ CMOS integration of sensor and electronics in an ASIC.

System Overview

**Detector:** SPAD (Single-Photon Avalanche Diode)

→ a p-n junction biased above its breakdown voltage at \( V_{BD} + V_{OV} \) to operate in Geiger-mode. \( V_{BD} \) is the breakdown voltage and \( V_{OV} \) the reverse bias overvoltage to operate the Geiger-mode.
- Single-photon sensitivity.
- Sub-nanosecond response time.
- Gated Mode operation.
- Compatibility with standard CMOS technologies (low-price).

**Excitation:** Laser Diode

- Fast pulsed Laser diode, to produce nanosecond FWHM (Full width at half maximum) pulses.
- Commercial Laser Diode (low-price).
- Simple Driver for Laser Diode (low-price).

**Time-to-Digital Converter**

- Offers a digital representation of the time an event occurred, in this case the time of arrival of the detected particle.
- Implemented with FPGA using counter + tapped delay line.
- 0 - 430 ns range with ~100 ps resolution.

**Microfluidic Chip**

- PDMS chip with one microchannel of 100 µm of width.
- Coverslip of 150µm thickness to seal the microchannel.

**System Characterization**

**Quantum dot:** are semiconductor particles, of only several nanometers in size, which absorb photons of light, then re-emit photons at different wavelength. The QdotB 605 (ThermoFisher) have been used to characterize the system.

**Pros**
- Long-term photostability.
- Tuneability of the emitting light with single-excitation light.
- Biconjugate – easy coupled to proteins, oligonucleotides, small molecules, etc.

The histogram is constructed with the FPGA and can then be read as convenient shown directly to display or send it via USB.

**SPAD integrate with the microchannel**

- No alignment required of the microchannel.
- Without Optics neither Filters keeping it simple and low-cost

**Easily adapted to the target label molecule**

and measurement needs (400nm - 1000nm)

**Biomedical Applications**

- Fluorescence Lifetime measurements
- Single Molecule Spectroscopy
- Single Molecule Detection
- Time-correlated single photon counting

**Other Applications**

- Particle counting
- Particle sizing

**Conclusions:** A compact system to measure fluorescence lifetime has been developed and tested on different fluorophores, showing a low-cost approach is feasible and opening the door to test it in a real application.

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