Aptamer-based protein biochip with a SPAD array time-resolved detection

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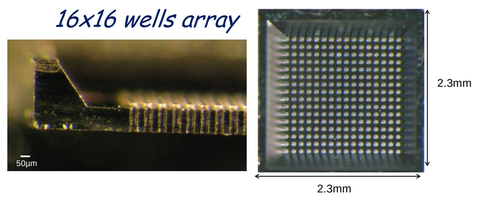
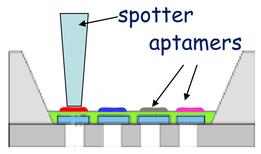
The idea is to develop a monolythic integrated system for the detection of proteins (in particular thrombin and vascular endothelial growth factor VEGF).
The key points of the system are:

1) The use of biofunctional layer based on DNA aptamers molecules
2) The use of the Single Photon Avalanche Diode (SPAD) as detector
3) The use of transparent microreactors array (MRA) on silicon substrate

16x16 wells array

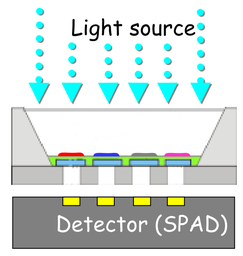


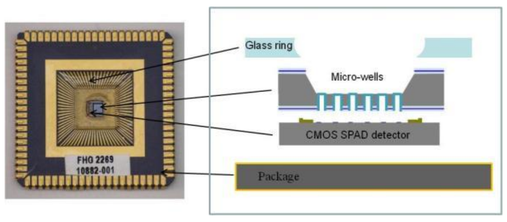
The micro-wells array is a matrix of micro-cavities closed with a thick membrane (2µm) of a $SiO_2/Si_3N_4/SiO_2$ multilayer. The membrane has the twofold advantage of being transparent and strong enough to act as a plug.



Primary DNA-aptamers are locally immobilized on the MRA using a spotter deposition

A light source excites the secondary fluorescent-labelled DNA-aptamer immobilized after protein recognition and a SPAD array, below the MRA structure, record the signal





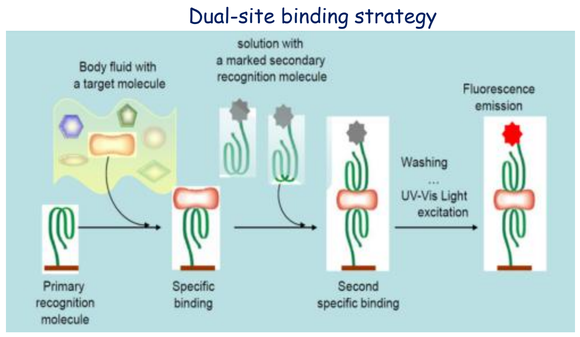
All the biofunctional, fluidic and detection layers will be integrated in a single package

Individual layers optimization

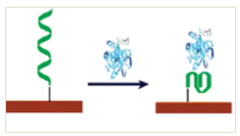
A: Biofunctional layer

Introduction:

Dual-site binding strategy



Detecting the biomolecular interaction with high sensitivity and reliability



Aptamers

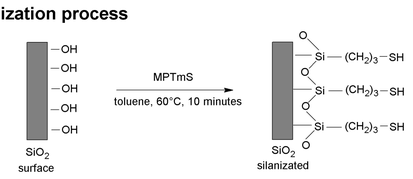
- in vitro selection (SELEX)
- high specificity and affinity
- high reproducibility and purity
- highly chemically stable
- great flexibility in design of novel biosensors

Biological targets

- Thrombin
- Vascular Endothelial Growth Factor (VEGF)

Results:

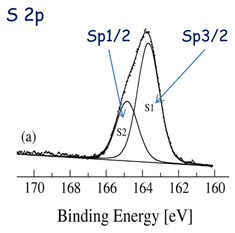
1) Silanization process



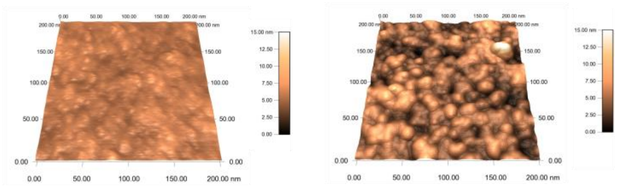
Elemental composition (atomic percentage) determined by XPS at 15° take-off angle. The standard error do not exceed 1-2% of the reported values.

Substrate (15 ° take off angle)	O 1s	C 1s	Si 2p	S 2p
Silicon oxide (after piranha)	49.2	16.8	34	-
After silanization	23.3	41.2	23.3	10.2

Sulphur core line (15° take-off angle)

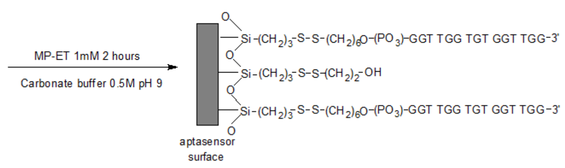


AFM images (200x200 nm²) in tapping mode on silicon oxide (left) and on mercapto-silanized silicon oxide (right). Z scale 0-15 nm

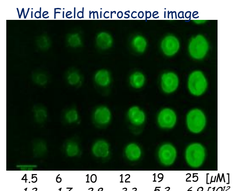


Substrate	Roughness (nm)
Silicon oxide	0.63 ± 0.05
After silanization	1.84 ± 0.12

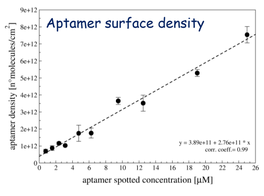
2) Aptamer immobilization and passivation



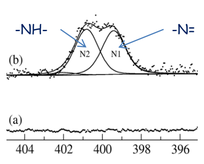
Wide Field microscope image



4.5 6 10 12 19 25 [µM]
1.2 1.7 2.8 3.3 5.2 6.9 [10^{12} molecules/cm²]

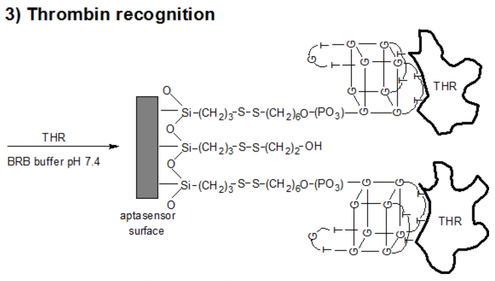


N 1s

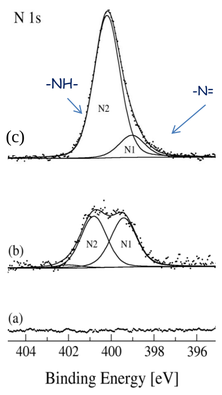


XPS. Nitrogen core line (15° take-off angle) after silanization (a) and after aptamer deposition from 10 µM thrombin aptamer binding solution (b)

3) Thrombin recognition

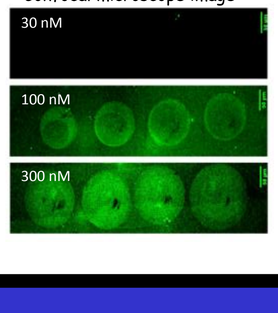


N 1s



XPS. Nitrogen core line (15° take-off angle) after silanization (a), after aptamer deposition from 10 µM thrombin binding DNA-aptamer solution (b) and 300 nM thrombin adhesion (c).

Confocal microscope image

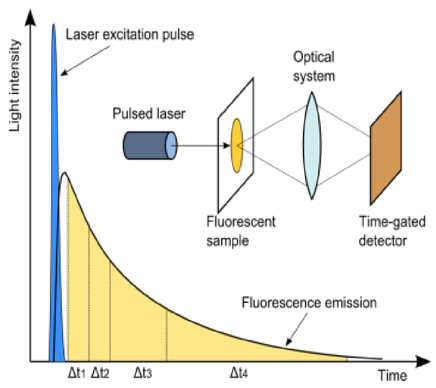


Substrate (15 ° take off angle)	O 1s	C 1s	Si 2p	S 2p	N 1s	P 2p
After silanization	23.3	41.2	23.3	10.2	-	-
After DNA-aptamer	19.9	53.6	15.4	7.5	2.5	1.1
After thrombin	20.7	54.5	11.1	5.7	7.3	0.7

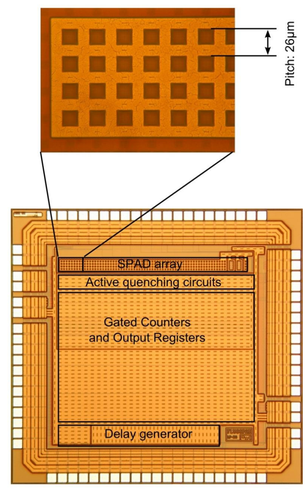
B: Detection layer

Introduction:

CMOS Visible Detectors: Time-gated Lifetime Measurement Technique



64-pixel linear SPAD array layout
4 SPADs x pixel
4 gated counters x pixel
Programmable gate width: 0.8ns – 10ns
SPAD array size: 0.1 x 1.6 mm2
Pixel pitch: 26um
Fill factor: 34%

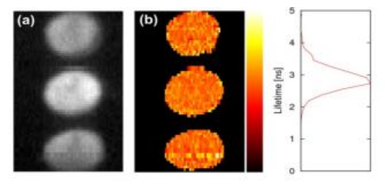


Results:

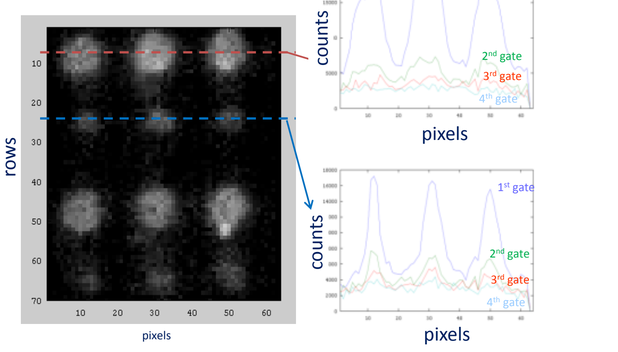
1- Fluorescent primary thrombin binding DNA-aptamer

The primary fluorescein-labelled thrombin DNA-aptamer was immobilized on flat samples at 20 or 2µM concentration using the spotter deposition. After washing and passivation step, the sample was analyzed by a linear 64x 4 linear SPAD array. Both fluorescence and lifetime analysis were performed

Raw data:
Scanning rate 1 row / 5 sec
Total measurement time: 6 min



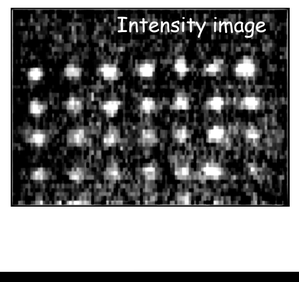
(a) Intensity image
(b) Lifetime image

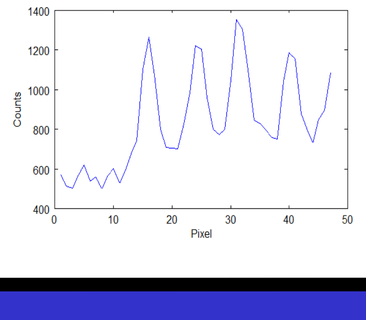


2 - VEGF detection using a secondary fluorescent DNA-aptamer

The primary VEGF DNA-aptamer (5'-OH-$(CH_2)_3$-S-S-$(CH_2)_3$-ATACCAGTCTATTCAATTGCACTCTGTGGGGGTGGACGGGCCGGGTAGA-3') was immobilized on flat samples using the spotter deposition. A 100nM solution of purified VEGF was incubated on the sample for 20 minutes in BRB buffer (EDTA 1 mM, $MgCl_2$ 1 mM, KCl 150 mM pH=7.4) and then recognized using a secondary fluorescein-labelled DNA-aptamer.
The fluorescence signals are then acquired by a linear 64x 4 linear SPAD array, scanning the sample.

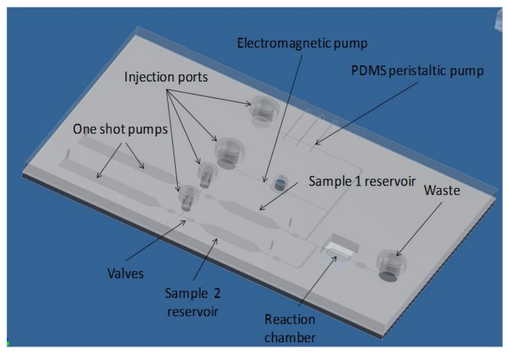
100 nM VEGF
Scanning rate 1 row / 10 sec
Total measurement time: 10 min



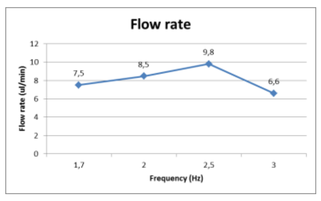


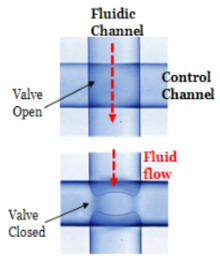
C: Fluidic layer

The final fluidic network for the lab-on-chip biosensor was designed and produced. The polydimethylsiloxane (PDMS) was selected as material for all microfluidic components



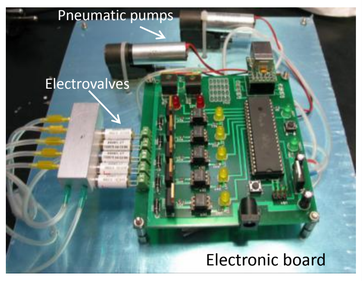
The peristaltic pump were tested using different open/close valves frequencies, and for many cycles to simulate the real work conditions





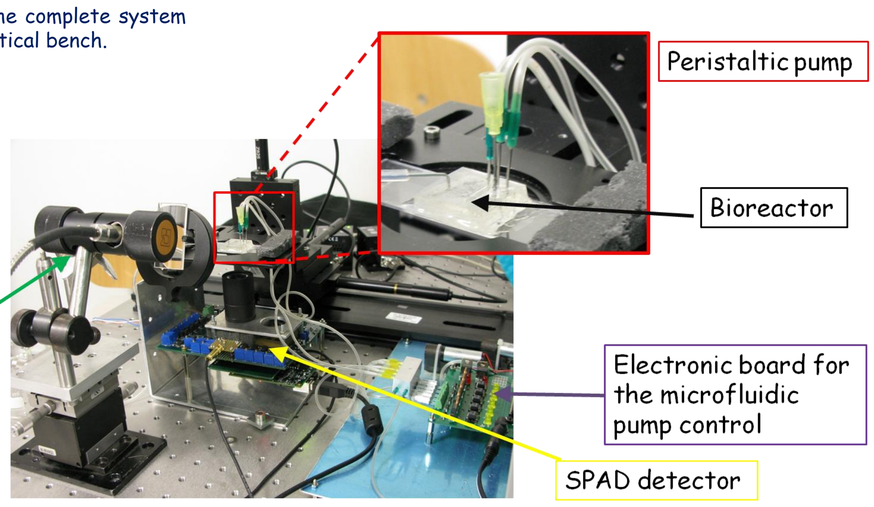
An electronic board was designed and developed for the control of the pneumatic pumps and electrovalves

The pump performance was also proved using complex biological solution, such as complete serum and entire blood, giving positive results

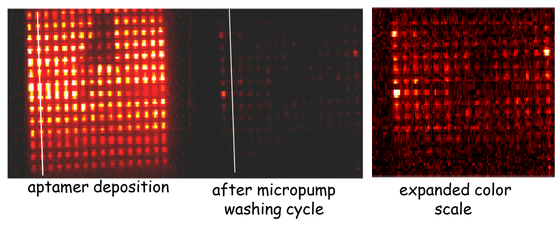


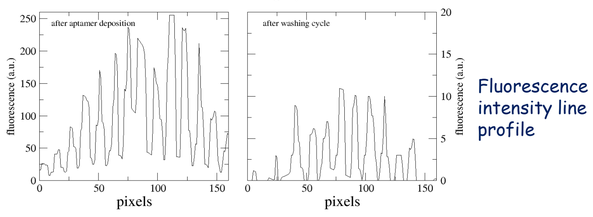
Integrated prototype development

A first prototype of the complete system was assembled on an optical bench.



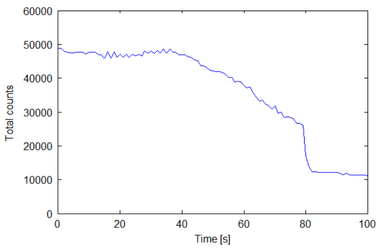
Testing of microfluidic pump performances, washing a functionalized microreactor with primary fluorescein-labelled DNA-aptamer and detecting the signal with the SPAD





Fluorescence intensity line profile

Fluorescence intensity decay measured on a column during the washing cycle



Future steps:

- ✓ new 32x32 pixel SPAD design
- ✓ fast disable-recharge (1ns) implemented to avoid laser's photon detection
- ✓ low cost pulsed source
- ✓ fully integration of the three layers