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On chip micro-extraction and real-time PCR with integrated SPAD optical fluorescence detection for nucleic acid analysis

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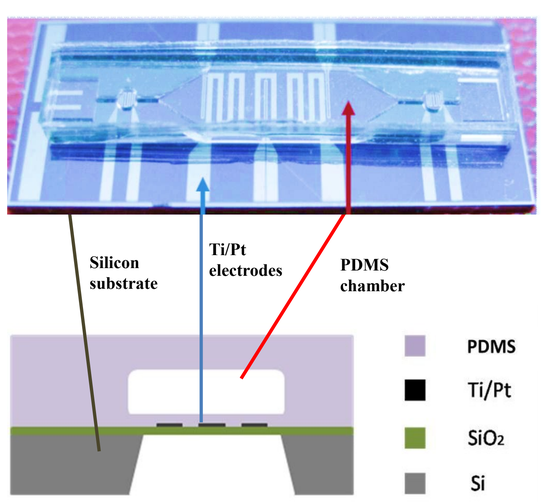
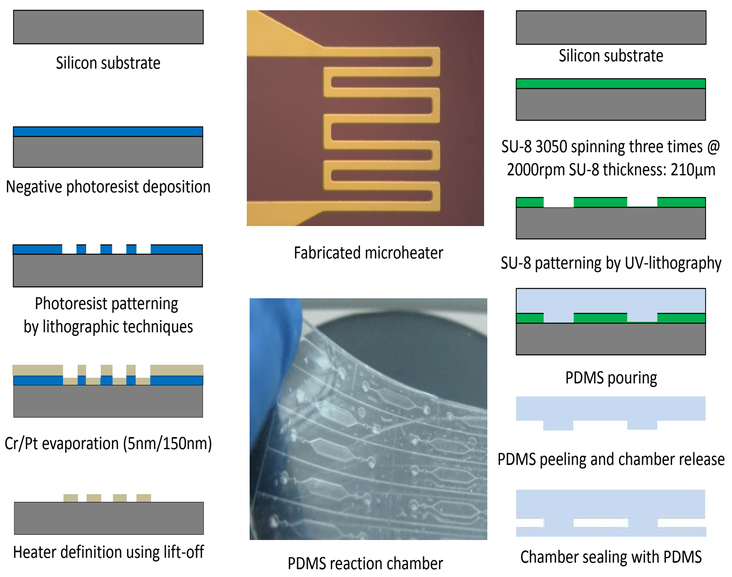
Abstract

A PDMS lab-on-a-chip for one step DNA isolation and real time-polymerase chain reaction (RT-PCR) has been designed, fabricated, and characterized for point-of-care clinical diagnostics. In addition, a module for on-chip optical detection based on SPAD - Single-Photon Avalanche Diode - detector has also been developed and used to monitor the presence of specific DNA polymorphisms possibly related to genetic diseases. Both the fluorescence intensity and the life-time of a specific probe were measured. DNA purification from whole blood and direct amplification on adherent DNA was successfully performed in the same PDMS chamber. RT-PCR was also demonstrated by using an optical reader connected to the chamber, integrating DNA purification, amplification and detection in the same microdevice.

PCR module design and fabrication



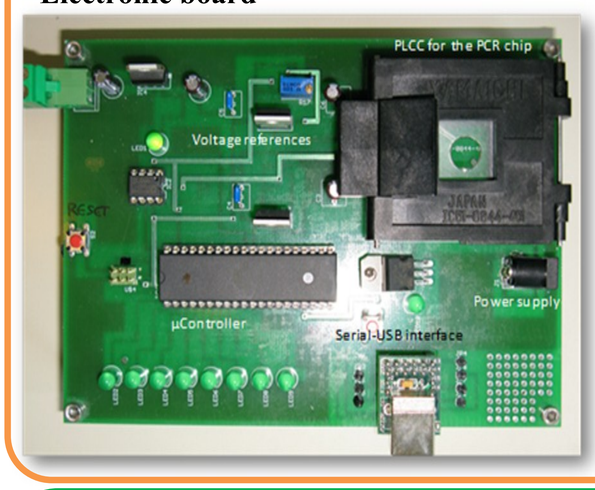


The integrated module for genomic DNA purification and PCR reaction is composed of two independent parts: a silicon substrate with embedded heater and thermometers and a PDMS chamber reactor as disposable element.

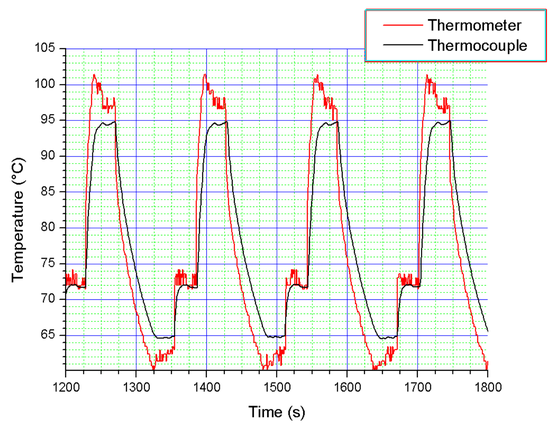
Temperature control

Thermal control has been implemented with a combination of feedback loop and forward control. The feedback loop was used to set the temperature on the silicon surface using the embedded thermometer.
The temperature cycles were optimized using a type K thermocouple and adjusting the set point (red line) in order to obtain a precision of ± 0.5°C with reduced heating time.

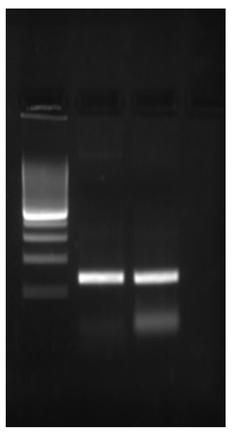
Electronic board



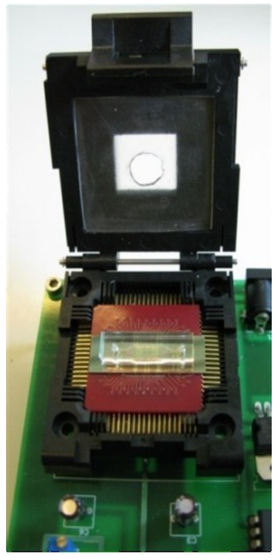
Temperature optimization



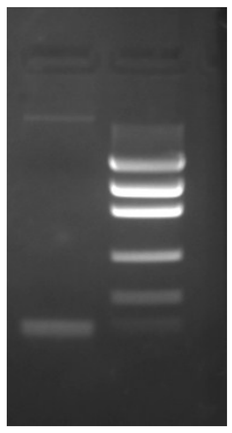
PCR module testing with sequences related to rheumatoid arthritis



274 bp



82 bp



2 independent PCR experiments with primer DRBamp-A / DRBamp-B on two parallel chips.

*PCR with DRBamp-A / primer 74a and template DNA typed as HLA-DRB1*0101.*

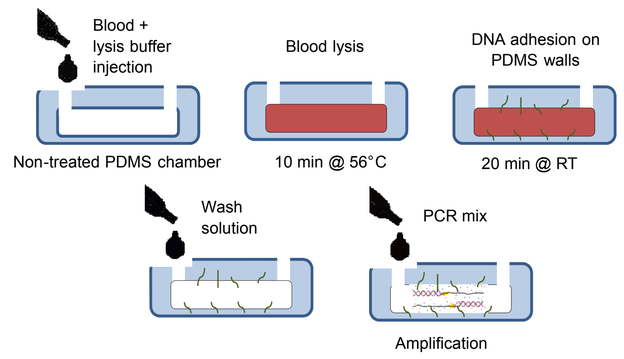
Primer name	Primer sequences (5' – 3')
DRBamp-A (5')	-8 CCCCACAGCACGTTTCTTG 11
DRB amp-B (3')	247 CCGCTGCACTGTGAAGCTCT 266
Primer 74a (3')	74 TCTTTCCAGCAACCGCAC 57
Probe 01	20 TAAGTTTGAATGTCATTTCTT 40

On-chip DNA purification and PCR

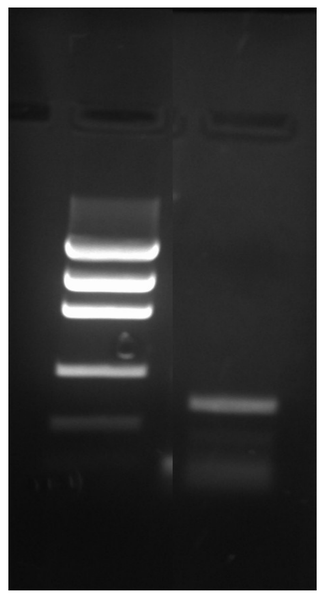
DNA purification & amplification:

- on-chip blood lysis (10' @ 56°C)
- DNA adsorption on non-treated PDMS surfaces (20 min RT)
- washes with ultrapure water
- PCR mix addition
- on-chip PCR

on the same chamber



Amplification from adsorbed DNA purified from whole blood lysed in the same chamber. Primer set: DRBamp-A / DRBamp-B.



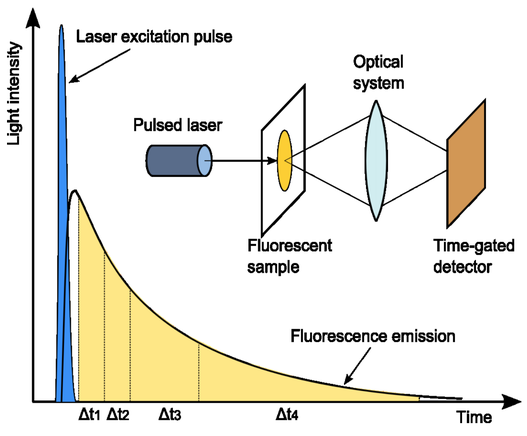
Setup for RT-PCR with SPAD detectors

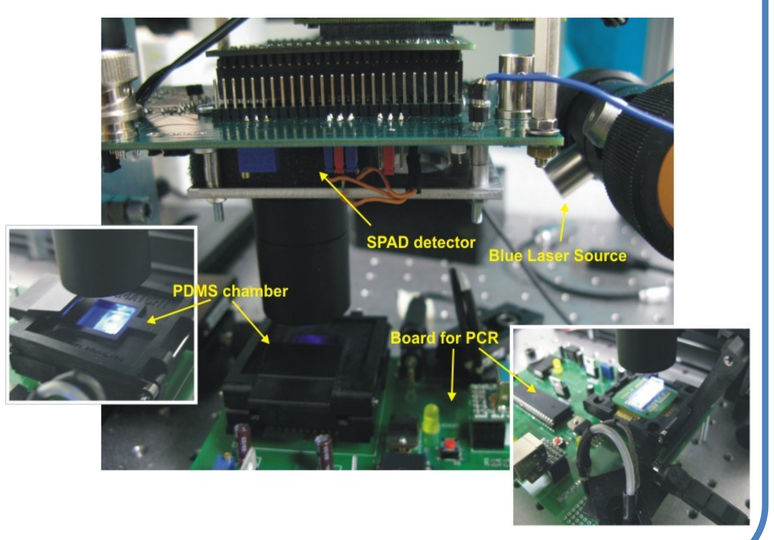
The **SPAD** is a reverse biased p-n junction with single photon detection capability and able to detect the arrival times of the photons.

- **High sensitivity**
- **Intrinsic digital conversion**
- **CMOS compatibility**
- **Time-gating approach**
- **Lifetime measurement**
- **High data throughput**
- **Modulated light**

SPAD Array parameters	
Technology	CMOS 0.35µm HV
Chip size	3.3 mm²
Pixel number	64
Pixel pitch	26µm
Fill factor	34%
Current consumption	200 mW @ 150 kfps
Dead time	200ns tpv
Dark count rate	1KHz tpv
Time resolution	160ps FWHM

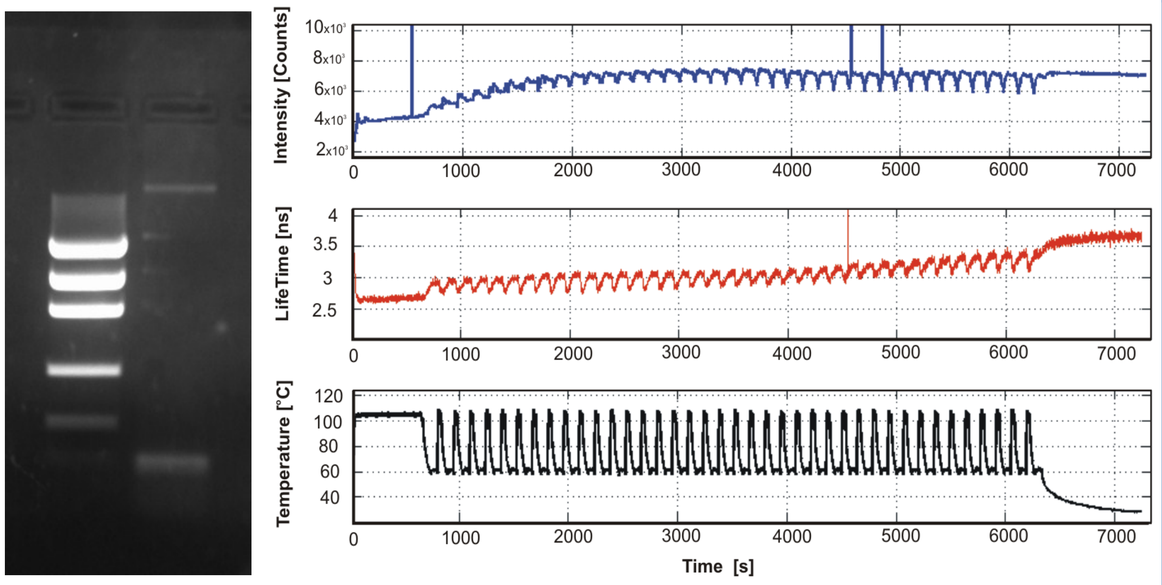
Time-gated Lifetime Measurement





RT-PCR with lifetime estimation

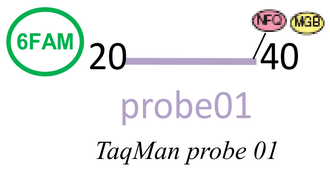
A real-time PCR has been performed using the proposed optical setup where the detected intensity of the fluorescence and life time estimation highlight that the amplification partially occurs, supporting the results obtained with the agarose gel.



Control of RT-PCR amplification in gel electrophoresis of the cycled PCR product.
*Primer set: DRBamp-A/primer 74a; DNA: HLA-DRB1*0101; TaqMan probe 01.*
The micro-reactor system combined with the SPAD detector was used to monitor the PCR in real time.

Exciting source of light

λ = 470 nm
Frequency = 40MHz
Pulse width = 80 ps
Average power = 200mW



TaqMan probe 01

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