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.

PCR module testing with sequences related to rheumatoid arthritis

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

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequences (5’ – 3’)</th>
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<tbody>
<tr>
<td>DRBamp-A (5’)</td>
<td>6 CCCGACACGCATTCTG 11</td>
</tr>
<tr>
<td>DRB amp-B (3’)</td>
<td>247 GCCTGGACTGAGGTAAAG 268</td>
</tr>
<tr>
<td>Primer 74a (3’)</td>
<td>74 TCTTCTGCCAACCAGCCGAC 57</td>
</tr>
<tr>
<td>Probe 01</td>
<td>20 TAAAGTTGGAAGTATGCTTCTG 40</td>
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Setup for RT-PCR with SPAD detectors

The SPAD is a reverse biased pn 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: 1.3 mm²
- Pixel number: 64
- Pixel pitch: 25µm
- Fill factor: 34%
- Current consumption: 200 mW @ 150 kfps
- Dead time: 200ns tpv
- Dark count rate: 1KHz tpv
- Time resolution: 100ps FWHM

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.

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-chip DNA purification and PCR

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

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*01, TaqMan probe 01. The micro-reactor system combined with the SPAD detector was used to monitor the PCR in real time.

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