A Viscometric Chip for DNA Analysis

Philipp Rust *, Damiano Cereghetti, Jurg Dual

Institute of Mechanical Systems, Department of Mechanical and Process Engineering, ETH Zurich
Tannenstrasse 3, CH-8092 Zürich, Switzerland

Abstract

This paper describes a hybrid silicon/plastic chip which is used to analyze DNA samples without the need of fluorescence or surface functionalization. The viscosity and density of the solutions to be tested are measured using a vibrating silicon beam immersed in the fluid. The sensitivity of the chip with respect to DNA concentration and length is analyzed, yielding a limit of detection of 0.5 μg/μl. We show that the sensitivity of the chip is statistically significant for PCR end-point detection. State-of-the-art on-chip detection methods of DNA solutions need optical devices or specially designed reactions. With the viscometric method proposed in this paper, these drawbacks are avoided.

© 2012 The Authors. Published by Elsevier Ltd. Selection and/or peer-review under responsibility of the Symposium Cracoviense Sp. z.o.o. Open access under CC BY-NC-ND license.

Keywords: viscosity; density; DNA; cantilever

1. Introduction

There have been tremendous efforts in the field of lab-on-chip devices to carry out a polymerase chain reaction (PCR) on microchips [1]. From an engineering point of view there has been a lot of optimization with respect to the reaction itself, such as temperature control and temperature cycling methods. Since the goal of such chips is often a “sample in answer out” device, the monitoring or evaluation of the reaction products is an important part. PCR product evaluation can be done with optical methods, such as fluorescent dyes. Another possibility is to use capillary electrophoresis. Both methods need more or less sophisticated optical devices or they are only partly suitable for lab-on-chip applications due to bulky equipment. We try to circumvent these disadvantages by using a vibrating cantilever which is incorporated into the fluidic chamber where the reaction takes place. The cantilever is not functionalized.

* Corresponding author. Tel.: +41 44 632 23 04; fax: +41 44 632 11 45.
E-mail address: ruest@imes.mavt.ethz.ch
but measures the fluid mechanic properties of the liquid it is surrounded with. The measurement is based on the fact that the resonance frequency and damping of the cantilever depend on the viscosity and density of the fluid. These values are dependent on the amount and conformation of the DNA which is in solution. The fabrication and the details of the circuitry have been described in detail earlier [2]. However, a brief overview of both will be given in the following.

2. Sensor Chip

The chip consists of three parts as shown in Figure 1 a): The sensor chip, where the cantilever is fabricated on, the PDMS lid and the heater chip. The assembly is mounted onto a printed circuit board (PCB) to facilitate handling and electrical connections. The fabrication of the cantilever chip, as illustrated in Figure 1 b), is based on a silicon on insulator (SOI) wafer (the thickness of the handle, buried oxide and device layer is 450 μm, 1 μm and 70 μm). After deposition of an oxide layer needed for electrical insulation, gold loops are deposited and structured with a lift-off process. In a subsequent step, the oxide layer is isotropically dry etched and the device layer is structured with a deep reactive ion etching (DRIE) process. In order to release the cantilevers, the handle layer and the buried oxide layer are etched from the backside, thereby also forming the cavity for the fluidic chamber. At the end a layer of silicon nitride is deposited in order to protect the gold layer from the liquid.

The lid on top of the chip is made of PDMS and attached to the chip with plasma bonding as shown in Figure 1 c). A silicon wafer, which is dry etched to depth of 200 μm serves as a casting mold. The lid has short fluidic channels and punched holes which serve as openings, where the liquid can be filled into the chamber with a pipette. The back side of the chamber is closed with a 300 μm thick silicon chip having a platinum resistive heater and a temperature sensor on the back side (see Figure 1 d)). The whole assembly is glued to the PCB and electrically connected by wire bonding.

Figure 1: a) Setup of the sensor, b) Fabrication of the cantilever chip, c) Fabrication of the PDMS lid with a silicon mold, d) Platinum heater and temperature sensor on the backside of the bottom lid.
The cantilever is brought into or near resonance using the Lorentz force by applying an alternating
current to the gold loops and with a permanent magnet. The induced current of the moving structure
serves as the readout signal. The resonance frequency and damping are measured with a gated phase
locked loop. This type of circuitry keeps the phase difference $\varphi$ between excitation and readout signal at a
fixed value, while switching between excitation and readout. The system is at resonance if $\varphi=0^\circ$. $Df$
which is a measure for damping is defined as the difference of the frequencies where $\varphi=\pm \Delta \alpha$.

3. Prefabricated DNA Solutions

In order to assess the chips for DNA analysis, solutions of 110 bp and 10 kbp long double stranded
DNA were measured at different concentrations ranging from 0.005 to 3.4 $\mu$g/$\mu$l for the 110 bp solutions
and from 0.26 to 133.4 $\mu$g/$\mu$l for the 10 kbp solutions. The buffer composition was 10 mM KCl in 50 mM
Tris-HCl, pH 7.5. The 110 bp sequence was as described by Tsortos et al. [3]. Each series was measured
three times at 23°C. Figure 2 shows the mean values for resonance frequencies $f_{res}$ and damping $df$ with
$\Delta \alpha=22.5^\circ$. The error bars indicate the minimum and maximum values. As expected, the resonance
frequency decreases and the damping increases with increasing concentrations. This is a combined effect
of viscosity and density change. The limit of detection for both solutions lies at approximately 0.5-1
$\mu$g/$\mu$l. This value is comparable to the one reached at the end of a PCR under ideal conditions. Figure 3
shows the same values with respect to copy number. The values for the 10 kbp solutions
increase/decrease at much lower copy numbers than the ones for the 110 bp solutions.

![Figure 2: Resonance frequency and damping for the two dilution series with respect to mass concentration.](image1)

![Figure 3: Resonance frequency and damping for the two dilution series with respect to copy number.](image2)
4. PCR Solutions

As a proof of concept, a PCR was carried out in a thermocycler and validated by agarose gel electrophoresis. Four samples were prepared consisting in one positive control (A1) and three negative controls (A2-A4) (see Table 1). The positive control A1 as well as the negative control A2 contained all the components required for PCR amplification except that in A2 the enzyme was added at the end of the cycling process. A3 contained no primers while in A4 genomic DNA was missing. Figure 4 shows the measured resonance frequencies for each sample. Looking at the resonance frequency, sample A1 can be clearly distinguished from the negative controls.

Table 1: Samples used to test the chips response to a PCR reaction. A1 is the positive control, whereas A2-A4 are negative controls.

|     | Polymerase | Primers | gDNA |
|-----|------------|---------|------|
| A1  | ✓          | ✓       | ✓    |
| (positive) |           |         |      |
| A2  | ✓          | ✓       | ✓    |
| (added at the end of the reaction) |           |         |      |
| A3  | ✓          | -       | ✓    |
| A4  | ✓          | ✓       | -    |

Figure 4: Resonance frequency for the PCR samples. A1 is the positive sample, A2-A4 are negative controls. Note the significant between A1 and A2.

5. Conclusions

The viscometric chip which is presented in this paper is capable of resolving concentrations of DNA in the range of an optimally working PCR reaction. As proof of concept, a PCR was run off-chip and tested on-chip, hence proving the validity of the concept.

References

[1] C. Zhang and D. Xing. Miniaturized PCR chips for nucleic acid amplification and analysis: latest advances and future trends. Nucleic Acids Res. 2007; 35(13): 4223–4237.
[2] P. Rust, I. Leibacher, J. Dual. Temperature Controlled Viscosity and Density Measurements on a Microchip with High Resolution and Low Cost. EUROSENSORS XXV, Athens, Greece, Procedia Engineering. 25, 2011
[3] A. Tsortos, G. Papadakis and E. Gizeli. The Intrinsic Viscosity of Linear DNA. Biopolymers. 2011; 95 (12): 824-832