

### 3-D MODELING AND SIMULATION OF FLUIDIC MICROSYSTEMS FOR BIOLOGICAL FLUIDS ANALYSIS

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**Abstract** — This paper describes a three-dimensional fluid dynamic model that is suitable for accurate simulation of on-chip liquid handling and mixing. The fluidic die (or mixer) is an integrated part of a biological microsystem for biological fluids analysis. It is designed for enabling a mixing process based on diffusion, which allows an easy-to-fabricate and low-cost mixer. The fabrication is based on planar technology using IC-compatible lithography. Computer simulations have been carried out to analyze the details of the flow and the diffusion in the mixer in order to derive the appropriate design criteria for the layout. The mixer has been fabricated using glass micro-machining with SU-8 techniques and wafer-to-glass bonding. Its performance is successfully demonstrated for serum albumin concentration detection.

**Key Words:** fluidic mixer, mixing by diffusion, biomolecules analysis, SU-8 microchannels.

## I INTRODUCTION

For diagnostic reasons patients are often subject to spectrophotometric analysis of their biological fluids. Usually, the samples need to be sent to a laboratory for analysis and the results become available after several hours, sometimes days. As a consequence a reliable diagnosis cannot be performed within the consultation time. The need for rapid and on-line measurements with low sample volumes has led to the development of microsystems with the fluidic, detection and read-out system integrated in a single-chip. The advantages associated with shrinking clinical analysis systems include: reduced sample size and costs, high degree of system integration, automation of measurements, short response time, improved analytical performance and laboratory safety [1].

The ability to mix two or more fluids thoroughly and in a reasonable amount of time is an essential requirement for any practical fully integrated (bio)chemical analysis systems in a single-chip. Microscale fluidic systems have distinctive

properties that result from their small dimension. First of all, the liquid flow is generally laminar, not turbulent. Secondly, diffusion in narrow channels is the only viable process for mixing fluids. Complete mixing of fluids in macroscopic devices generally involves the generation of turbulence, using three-dimensional flow structures or mechanical actuators for stirring. However, as the fabrication of microfluidic devices is in a planar lithographic design environment, mechanical actuators or three-dimensional complex structures should be avoided. Consequently, a passive mixer should be the alternative. In passive mixers, the mixing is caused by diffusion that is due to the relatively long transit time in the channels at small flow rates. Unfortunately, when large molecules with low diffusion coefficients ( $< 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ ) are to be mixed, this approach requires either extremely long channels ( $> 1$  meter) or a decrease in flow rate to achieve sufficient mixing, as the interaction time at a given length would increase [2]. This work aim on the fabrication of a fully integrated biological microsystem that includes the fluidic die (the mixer) for spectrophotometric measurement of serum albumin concentration.

## II BACKGROUND CONCEPTS

### II.1 ALBUMIN ANALYSIS

In clinical diagnostics, spectrophotometry by optical absorption is often used to determine the concentration of a particular biomolecule in biological fluids samples [3]. The measurement is based on colorimetric detection by the optical absorption in a part of the visible spectrum defined by the biomolecules present in the sample. However, many of the analytes (substance being

analyzed) of interest for clinical analysis do not have chromophores that absorb light in a useful part of the visible range. Specific chemical reactions are available (reagents) to transform these analytes into colored products that do have adequate absorbance. In addition, the biomolecules concentration is measured by using a mixture of a reagent with an analyte sample.

This is the case of serum albumin concentration detection, in which it has been used the bromocresol green (BCG) reagent, commercially available from Sigma Aldrich [4]. When it reacts with albumin the mixture produces a blue green color with an absorbance maximum peak at  $\lambda = 628$  nm. The intensity of this color is directly proportional to the albumin concentration in the sample. The reagent manual procedure requires only a gentle inversion of the cuvette that contains the mixture. This is enough to completely mix the two liquids (due to the high diffusion coefficients of the serum albumin in the BCG reagent [4]). Therefore, the liquids to be mixed have good characteristics for being mixed using diffusion. Thus, a passive mixer, which is easy-to-fabricate compared to an active mixer, is adequate.

## II.2 FLOW CHARACTERISTIC

Typically, the liquid flow in microfluidic channels is laminar. The turbulence flow regime is achieved at large Reynolds number, defined as

$$Re = \frac{\rho V \delta}{\mu} \quad (1)$$

where  $\rho$  is the specific mass,  $V$  is the velocity,  $\delta$  is the length scale and  $\mu$  is the dynamic viscosity. For a rectangular cross section, the length scale is the hydraulic diameter. The appropriate length scale, typically the channel length, will in general be smaller than  $500 \mu\text{m}$ . Assuming the highest velocity to be experienced for on-chip flow is one die length per second ( $V = 10 \text{ mm s}^{-1}$ ) and, if water is used,  $Re \approx 5$ . Thus the flow is laminar, and it can be discounted turbulence as an available mixing mechanism. This obviates the use of barrier-fields, complex geometries and severely limits the usefulness of mechanical actuators [5].

## III CFD MODEL

The size and shape of microfluidic devices limit the usefulness of diffusion as a sole mechanism for

mixing. As it is difficult to mix two fluids in a planar device, the length over which diffusion must act is defined by the in-plane dimension of the fluid channel. The reason for the diffusion is the large gradient of the concentration of fluid molecules, which exists when two different liquids have a common interface. Using Fick's equation, the diffusion mixing time scale  $t_D$  can be defined as [2]

$$t_D = \frac{L^2}{D} \quad (2)$$

where  $L$  is the relevant mixing length, and  $D$  is the Fickian diffusion coefficient.

A computational fluid dynamic (CFD) model that takes into account the diffusion coefficient was established and used to simulate the on-chip flow distribution and diffusion (see Figure 1). It has two inlets, one for the reagent (inlet 1) and the other for the sample (inlet 2). The liquids are injected using a syringe pump in order to minimize the appearing of air bubbles. Starting at the U-junction the liquids diffuse while travelling down the main channel to the detection chamber. The channels are  $500 \mu\text{m}$  wide and  $500 \mu\text{m}$  deep, the main channel is  $70 \text{ mm}$  long and has 6 U-turns. Finally, the square detection chamber is  $2 \text{ mm}$  wide,  $500 \mu\text{m}$  deep and  $2 \text{ mm}$  long. The absorbance measurement is performed when the mixture is in the detection chamber, thus its high depth is crucial for the optical absorption measurements. Under the detection chamber there are photodetectors (16, each with  $500 \mu\text{m} \times 500 \mu\text{m}$ ) used to read the intensity of the light transmitted through the mixture. The area of those optical elements sets the size of the detection chamber.

Numerical analysis was performed using Ansys Flotran with the Flotran CFD option, which enables the design of complex microfluidic devices by modeling of the fluid flow fields [6]. The mixer was modeled by solving the Navier-Stokes equation for the velocity and pressure fields. The steady-state velocity field was subsequently used for solving the coupled species transport equations (reagent and serum). The concentration of these two species was assumed to be dilute and thus the properties of the carrier to be constant. The species have a low molecular weight and a high diffusion coefficient. The above equations were solved using a fully three-dimensional FE-based CFD engine.

#### IV SIMULATION RESULTS

The evaluation of the mixing process was carried out using the Sigma diagnostic kit (BCG reagent) and standards of serum with several albumin concentrations. From the material data sheet of the two liquids, fluid properties, such as incubation time, chemical formulas, molecular weight, solubility, density, diffusivity and thermal conductivity were derived. Others, such as the viscosity, are obtained by measurements at the mechanics department.

The mixing process for an albumin concentration of 4 g/dl and a flow rate of  $0.22 \mu\text{l s}^{-1}$  is shown in Figure 1. The normal range concentration in a human's serum is 3.8 g/dl to 5 g/dl. 20  $\mu\text{l}$  of reagent enters the mixer by inlet 1 and 0.4  $\mu\text{l}$  of serum sample by inlet 2. These volume quantities assure that even with the lowest albumin concentration, sufficient molecules for an accurate analysis are available (1 molecule of albumin occupies  $2.4 \times 10^{-13} \text{ nl}$ ).

The liquids were introduced at the inlets and fed through the mixer to the outlet. The liquid pressure in the outlet was kept at zero. Driven by the liquid pressure, liquids come across at the U-shape intersection. Incompletely mixed zones are seen in the mixer (see Figure 1). After the 5<sup>th</sup> U-turn the mixing is complete and homogeneous.

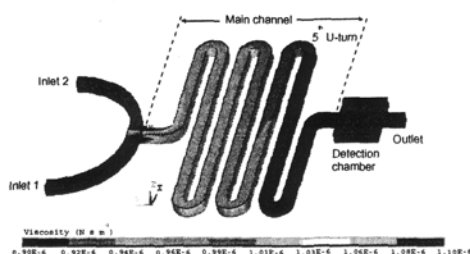


Figure 1. Simulation flow of the diffusion mixing process.

Due to the low fluidic flow rate the fluid flow is laminar, but a secondary rotational flow is generated in the U-turns of the mixer due to the centripetal acceleration, promoting a homogeneous mixing at the end of the main channel.

Other albumin concentrations in a range from 1 g/dl to 5 g/dl, comprising the range of normal and abnormal values in a human's serum, were simulated. The simulation results are similar to the ones shown in Figure 1. The mixing was completed at about the 5<sup>th</sup> U-turn, depending

whether or not the albumin concentration exceeds the value used in Figure 1. It can be concluded that for a flow rate  $\leq 0.22 \mu\text{l s}^{-1}$ , a homogeneous mixing is obtained on the way down the main channel. This basic geometry layout fulfils the requirements for albumin analysis in a human's serum since the mixing between the analyte and the reagent is completed before reaching the detection chamber. Increasing the flow rate from 0.22 to  $1.5 \mu\text{l s}^{-1}$ , results in incomplete mixing at the detection chamber (see Figure 2). The transit time is clearly insufficient for thorough mixing by diffusion. This flow rate results in a transit time of 11.7 s (less than the minimum of 60 s demanded by the reagent manual procedure).

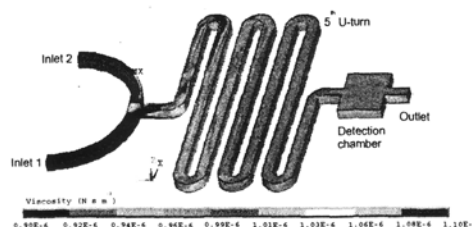


Figure 2. Simulation flow showing the incomplete mixing.

Simulations are being carried out in order to reduce the channel width. The channel depth and the rectangular shape are the most important characteristic of the device and should be kept since the detection is based on optical absorption measurements. A minimum lightpath of 500  $\mu\text{m}$  is needed to achieve reasonable measurements sensitivity.

#### V THE FLUIDIC DIE

An integrated mixer suitable for the measurement of albumin concentration in serum was fabricated according to simulation results (see Figure 3). The liquids enter and exit the device through inlets and outlet holes drilled in the top glass wafer. The flow in the system is parallel to the glass substrate. The channels are fabricated using a layer of photoresist SU-8 deposited on the glass substrate. This epoxy-based material offers good properties, such as high mechanical strength, good adhesion on many different substrate materials and biocompatibility. The SU-8-based fabrication is a low-cost process, UV lithography semiconductor compatible and the mask to be used for patterning the microchannels is a regular transparency foil (like the one used in printed circuit boards). Moreover, SU-8-based

processing enables the fabrication of rectangular shape and deep microchannels with very low sidewall roughness, which is suitable for optical absorption measurement [7].

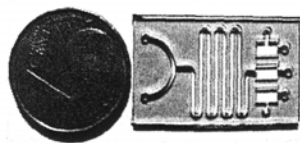


Figure 3. A photograph of the fabricated fluidic die.

## VI EXPERIMENTAL RESULTS

Figure 4 shows the measured transmittance response for serum albumin concentrations from 1 g/dl to 5 g/dl. The transmittance is defined as  $T = I/I_0$ , where  $I$  is the measured photodiode current for each solution and  $I_0$  the measured photodiode current of the reagent. The transmittance at  $\lambda = 628$  nm as a function of the different albumin concentrations is shown in Figure 5.

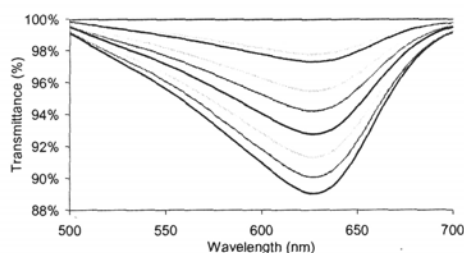


Figure 4. Measured transmittance spectra for different albumin concentrations. From top to bottom curve: reagent, 1, 1.25, 2, 2.5, 3, 4, 4.5 and 5 g/dl.

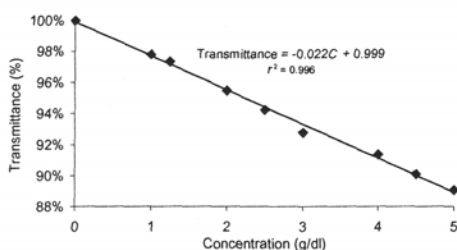


Figure 5. Transmittance at  $\lambda = 628$  nm for the measured albumin concentrations.

The results allow concluding that the simulated and the fabricated mixer is suitable for the required application, once they agree with macroscopic measurements performed in the state-of-the-art laboratory equipment.

## VII CONCLUSIONS

A fluid dynamic model was developed to simulate the on-chip mixing process of the BCG albumin reagent with serum samples. The simulations and the experimental results show that a homogeneous mixing is obtained by diffusion only, provided that the low flow rate is sufficiently low ( $< 0.22 \mu\text{l s}^{-1}$ ), so that the transit time through the channels is at least 60 s. This approach allows an easy-to-fabricate and low cost mixer by planar lithographic fabrication technology. Moreover, the mixer can be a disposable die, which minimize the cost associated with cleaning of the microchannels and avoids the contamination between analyses. Simulations for other biomolecules detection (such as bilirubin, glucose, total protein, etc.) and also other biological fluids are on-going.

## ACKNOWLEDGMENTS

The authors wish to thank Peter Turmezei from the Dept. of Microelectronics, Delft University of Technology, The Netherlands, for his help with SU-8 processing. Support for this research was provided by the Engineering School (IN2TEC) and Algoritmi Centre of University of Minho, Portugal.

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