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

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***Abstract***
*This paper describes a fluid dynamic model that is suitable for accurate simulation of the 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 driven by diffusion, which allows an easy-to-fabricate and low-cost mixer. The fabrication is based on planar technology using OIC-compatible lithography, which yields narrow channels in the vertical direction. 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 and wafer-to-glass bonding. The performance is demonstrated using the fast-reaction of infinity™ uric acid reagent with urine samples.*

***Key Words: Microfluidic system, CFD, fluidic mixer, fluidic channel, biosystem, uric acid analysis.***

## I. INTRODUCTION

Spectrophotometric analysis of a sample is one of the most commonly used analytical techniques for determining the concentration of a particular component in a biological fluid [1]. The need for rapid and on-line measurements has led to the development of biosystems (biological microsystems) with the fluidic, detection and readout systems integrated in a single-chip. The advantages associated with shrinking clinical analysis systems include: improved efficiency with respect to sample size, integration, automation, response times, laboratory safety and cost.

The ability to mix two or more fluids thoroughly and in a reasonable amount of time is essential requirement for any practical fully integrated chemical analysis systems in a single-chip. Microscale fluidic systems have distinctive properties that result from their small dimension. First of all, liquid flow is generally laminar, not turbulent. Secondly, diffusion in narrow channels is practically the only process for mixing fluids. The complete mixing of fluids in macroscopic devices is generally performed using turbulence, three-dimensional flow structures or mechanical actuators. However, as the fabrication of micro-fluidic devices is in a planar lithographic design environment, mechanical actuators or three-dimensional complex structures should be avoided.

Mixers can be classified into two categories: active and passive. Active mixers drive the mixing process by applying an external force in conjunction with the force that is transporting the fluid. 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 ($< 10^{-7}$ $cm^2$ $s^{-1}$) are to be mixed, this approach requires either extremely long channels (> 1 meter) or an increase in flow rate to achieve sufficient mixing [2].

This work aims on the fabrication of a fully integrated biosystem for uric acid analysis in urine that includes the fluidic die (the mixer).

## II. THEORETICAL ANALYSIS

### II.1. BACKGROUND OF URIC ACID ANALYSIS

The application of this biosystem is in uric acid analysis in which the detection is made by optical absorption in a well-defined wavelength of the visible spectrum. However, many of the analytes[1] 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, such as the infinity™ uric acid reagent used for uric acid measurement in urine samples, to transform these analytes into colored products that do have adequate absorbance. In this colorimetric

[1] An analyte is the substance (element, ion, compound or molecule) being analyzed.

assay[2] the absorbance of the mixture (reagent + analyte) is proportional to the concentration of the original colorless analyte [1]. The measurement method is commercially available from Sigma Aldrich. It is an enzymatic, colorimetric (as required) and endpoint assay. It is enzymatic because it uses enzymes as reagents to perform the reaction. Enzymes do not change the equilibrium of reactions; they only influence the speed with which equilibrium is attained. The series of reactions involved in the assay system is as follows: (1) uric acid is oxidized to allantoin by uricase with the production of $H_2O_2$; (2) the peroxide reacts with 4-aminoantipyrine (4-AAP) and TBHB in the presence of peroxidase to yield a quinoneimine dye. It is a colorimetric method because a light red color is produced with an absorbance maximum at 500 nm, when uric acid is bonded to infinity™ reagent. The intensity of the color produced is proportional to the uric acid concentration in the sample. It is endpoint (quantitative conversion of analyte to product) because it uses an excess enzyme concentration and allows the reaction(s) to proceed until ≥ 99% of the analyte is converted to the desired product. Like that the product is directly detectable [3].

The manual procedure for the reagent used 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 involved molecules). 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. KINETICS AND DIFFUSION

The assay for the analysis reported in this paper is a kinetic assay. In a kinetic assay the mixture concentration changes with time. Consequently, the measurements must be done after the incubation time and before there is any reverse reaction (between 5 and 15 minutes for the reported assay).

The reaction between the reagent on the particle surface and the analyte involve binding the molecule in solution to the reagent on the surface. The reaction rates are given by:

$$V_a = K_a[A][C]\ ,\ V_d = K_d[AC] \qquad (1)$$

where $A$ is the reagent, $C$ is the analyte, $AC$ is their complex on the particle surface, $K_a$ and $K_d$ are the so called rate constants of the association and dissociation, $V_a$ is the reaction rate of the association of $A$ and $C$, and $V_d$ is the reaction rate for the dissociation of $AC$. The letters in brackets symbolize the concentration of the molecules. A reagent-coated particle depletes the liquid that surrounds it of analyte and therefore diffusion of analyte towards the particle takes place (Fig. 1) [4].

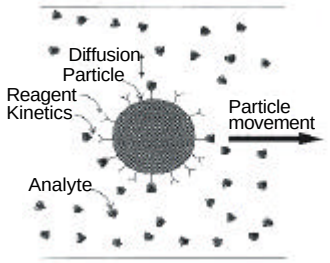


*Fig. 1. Overview of the activities around the reagent-coated particle. The particle moves with a certain velocity through the sample while the immobilized reagent picks up analyte (governed by the kinetics).*

### II.3. LAMINAR FLOW

Typically, the liquid flow in microfluidic channels is laminar. Turbulence occurs in flows characterized by high Reynolds number, defined as

$$Re = \frac{rVD}{n} \qquad (2)$$

where $r$ is the density, $V$ is the velocity, $D$ is the length scale and $n$ is the viscosity. The appropriate length scale, typically the channel length, will in general be smaller than 500 µm. Assuming the highest velocity to be experienced for on-chip flow is one die length per second ($V$ = 10 mm $s^{-1}$), it can be found an upper bound on the Reynolds number of $Re$ = 5, with typical values being much lower. As turbulence in channel flow occurs only for $Re$ > 2000, it can be expected on-chip flows to be 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. COMPUTATIONAL FLUID DYNAMICS (CFD) MODEL

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

[2] An assay is a method for the determination of the level of a given analyte in a given sample or set of samples.

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, a diffusion mixing time scale $T_D$ can be defined [3]

$$T_D = \frac{L^2}{k} \quad (3)$$

where $L$ is the relevant mixing length, and $k$ is the Fickian diffusion coefficient ($k$ = 163 µm$^2$ s$^{-1}$ for uric acid in the infinity$^{TM}$ reagent [1]). $L$ = 70 mm, results in $T_D$ = 30 s., which is relatively slow. However, it is adequate when also considering the incubation time of 300 s. Usually, the kinetics of colorimetric detection assays for biological fluids analysis result in an incubation time of several minutes, depending on the analysis [1].

A fluid dynamic model that takes into account the diffusion coefficient was established and used to simulate the on-chip flow distribution and diffusion. The basic geometry of the simulated device is shown in Fig. 2. The reagent and the analyte sample are introduced in the U-shaped channel via inlets 1 and 2, respectively. Inlet 1 is longer due to the reagent and analyte sample volume ratio and flow properties. Starting at the U-junction the liquids diffuse while traveling down the main channel to the detection chamber. The channels are 200 µm wide, the main channel is 70 mm long and has 6 U-turns. Finally, the square detection chamber has a 1.1 mm side. The absorbance measurement is performed when the mixture is in the detection chamber. An array of 16-photodetectors, placed under the detection chamber, is used to read the intensity of the light transmitted through the mixture. Its area of 1×1 mm sets the size of the detection chamber.

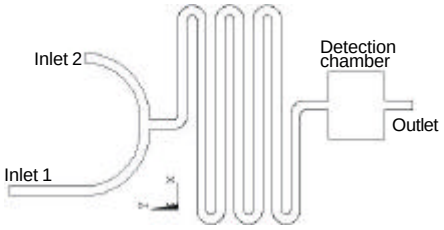


*Fig. 2. Layout of the fluidic die (mixer).*

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 urine). 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 two-dimensional FE-based CFD engine.

## IV. SIMULATION RESULTS

The evaluation of the mixing process was carried out using the Sigma diagnostic kit (infinity$^{TM}$ uric acid reagent) and standards of urine with several concentration of uric acid. From the material safety data sheet of the two liquids, fluid properties, such as incubation time, chemical formulas, molecular weight, solubility, density and conductivity were derived. Others, such as the viscosity, are obtained by measurement at the mechanics department of the University of Minho.

The mixing process for a uric acid concentration of 40 mg/dl and a flow rate of 2.3 mm s$^{-1}$ is shown in Fig. 3. The normal and abnormal range concentrations in a human's urine are 27 mg/dl to 54 mg/dl and 17 mg/dl to 67 mg/dl, respectively. 9.5 µl of reagent enters the mixer by inlet 1 and 0.19 µl of urine sample by inlet 2. These volume quantities assure that even with the lowest uric acid concentration (5 mg/dl, which is lower than the range of abnormal values) sufficient molecules for an accurate analysis are available (1 molecule occupies 1.14×10$^{-5}$ pl of solution).

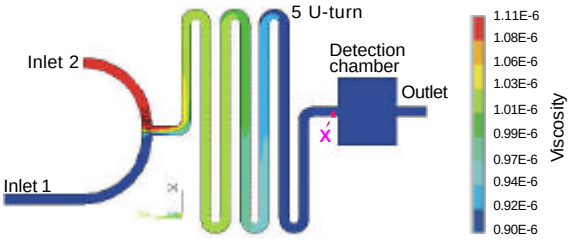


*Fig. 3. Simulation flow of the diffusion mixing process. The graphic displays the viscosity profile of the model.*

The liquid were loaded and flowed from inlets to outlet. The liquid pressure in the outlet was kept at zero. Driven by the liquid pressure, liquids come across at the U-shape intersection. Due to the low

fluidic flow rate (2.3 mm $s^{-1}$) the fluid flow is laminar with low Reynolds number (upper bound on the Reynolds number is 1.13). Incompletely mixed zones are seen in the mixer (see Fig. 3). After the 5th U-turn the mixing is complete and homogeneous. Fig. 4 shows the profile of the liquid flow in the main channel (location **X** in Fig. 3). The resulting path plot shows that an almost fully developed laminar flow profile has established. The curve is relatively symmetric and parabolic.

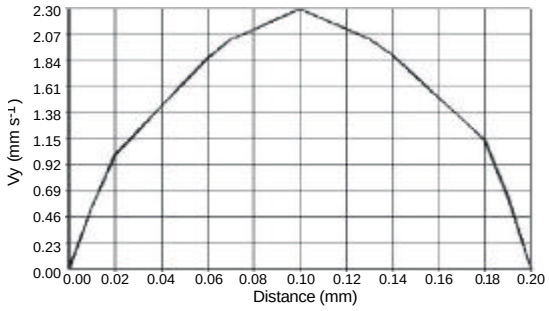


*Fig. 4. Flow profile.*

Other uric acid concentrations in a range from 5 mg/dl to 120 mg/dl, comprising the range of normal and abnormal values in a human's urine, were simulated. The simulation results are similar to the ones shown in Fig. 3. The mixing was completed at about the 5th U-turn, depending if the uric acid concentration is less or more than in Fig. 3, respectively. It can be concluded that for a flow rate ≤ 2.3 mm $s^{-1}$, a homogeneous mixing is obtained on the way down the main channel. This basic geometry layout fulfills the requirements for uric acid analysis in a human's urine since the mixing between the analyte and the reagent is completed before to reach the detection chamber.

Increasing the flow rate from 2.3 to 6 mm $s^{-1}$ (a syringe pump drove the fluids), results in incomplete mixing at the end of the channel (outlet). The transit time is clearly insufficient for thorough mixing by diffusion (see Fig. 5). This flow rate results in a transit time of 11.7 s (less than the minimum required of 30 s).

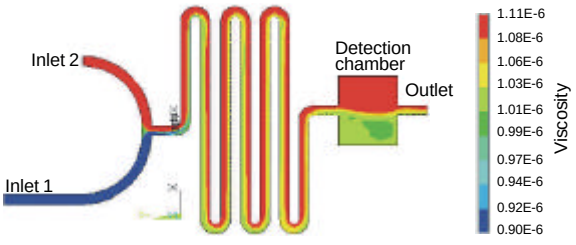


*Fig. 5. Simulation results for 5 mg/dl uric acid concentration in urine.*

## V. THE PROTOTYPE INTEGRATED FLUIDIC DIE

A perspective cross-section of the prototype integrated fluidic die is shown in Fig. 6. 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.

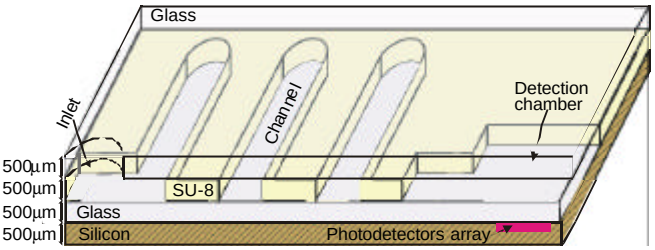


*Fig. 6. Perspective cross-section drawing of the prototype integrated fluidic die.*

## VI. CONCLUSIONS

A fluid dynamic model was developed to simulate the on-chip mixing process of the fast-reaction of infinity™ uric acid reagent with urine samples. The simulations show that a homogeneous mixing is reached, caused by diffusion, at low flow rates (< 2.3 mm $s^{-1}$), provided that the transit time through the channels is at least 30 s. This approach allows an easy-to-fabricate and low cost mixer by planar lithographic fabrication technology. A 3-D modeling is being carried out for further simulations.

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