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## LETTERS

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## Study of a chaotic mixing system for DNA chip hybridization chambers

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Numerical simulations of a micromixing system based on chaotic advection for improved deoxyribonucleic acid (DNA) chip hybridization are presented. To attain best chip performance, homogeneous dispersion of DNA molecules throughout the chamber in which the chip is placed is of primary importance. Poincaré sections of a simple time-periodic flow, based on numerical simulations of the flow, are compared with visualizations in a scaled-up experiment, with good agreement. The influence on mixing efficiency of varying the period of the flow at fixed volume flow rate is studied and a trade off is found between the absence of regular islands and a small enough total sample volume. The results illustrate the potential for optimization of such devices based on numerical flow simulations. © 2004 American Institute of Physics.

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The DNA chip has become one of the core technologies in genetics research. A *DNA chip* is composed of an array of biological probes, such as arrays of oligonucleotides with predetermined sequences, fixed to a solid surface. With improvements of miniaturization techniques, it is possible to have hundreds of thousands of probes on a chip of 1 cm width or less. There are many different methods for making and reading such a chip, but this lies outside the scope of the paper (see Ref. 1 for an overview). In the process of genetic analysis, the array is exposed to labeled DNA samples (with fluorescent markers, for instance). When complementary sequences combine together, the chip is said to be *hybridized*. That is to say (in theory): the labeled DNA samples are hybridized specifically to their complementary biological probes, and are not hybridized to noncomplementary probes. Thus, using a fluorescence technique, some spots on the array fluoresce and others do not, leading to the determination of the chemical composition (the genetic sequence) of the DNA samples.

Hybridization requires that complementary molecules encounter each other at some point in time. This may be achieved by pure molecular diffusion of DNA in the solution. In that case it is possible to estimate the typical diffusion

time: for a chip of width  $\ell = 10$  mm, with a diffusion coefficient  $D$  of DNA in water estimated to be about  $10^{-9} - 10^{-10}$  m<sup>2</sup> s<sup>-1</sup>, the typical diffusion time is

$$\tau_{\ell} = \frac{\ell^2}{D} \approx 30 - 300 \text{ h.} \quad (1)$$

Such a long characteristic time is, of course, incompatible with routine use of this kind of technology. It is therefore necessary to create a flow to improve dispersion across the chip. The DNA chip is placed in a “hybridization chamber” of width  $L$  and height  $h$ , and a flow is produced inside the cavity. However, in such a small device, only creeping flows are available, leading us to explore the possibilities offered by chaotic advection.<sup>2-4</sup>

It is of course of paramount importance to make sure that the DNA samples can potentially react *equally* with all spots of the array. However, in a chaotic flow, depending on the flow parameters, regular regions may coexist with chaotic ones, leading to nonuniform concentration inside the chamber. Since the hybridization reaction depends on concentration, this would lead to poor results from the chip.

To overcome this problem, we propose and study the mixing efficiency of a chaotic stirring protocol based on alternating injections. This protocol combines time-periodic forcing<sup>5,6</sup> and local three dimensional effects.<sup>7-9</sup> Independen-

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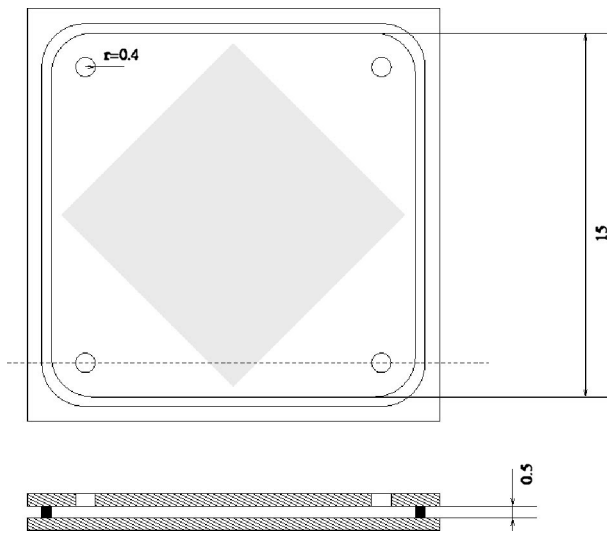


FIG. 1. Schematic view of the hybridization chamber. All lengths in mm. The DNA chip is symbolized by the central gray square.

dently of our work, McQuain *et al.*<sup>10</sup> have recently presented an experimental study of a micromixing device for chip hybridization. Their proposed chaotic mixer is very similar to ours, but in contrast with their work, the objective of the present study was to carry out a complete fluid mechanical analysis based on numerical flow simulation and determination of the particle trajectories. The results, which were validated using a scaled-up experiment, allow quantification of mixing efficiency and its variation as a function of the flow parameters.

A schematic view of a typical DNA hybridization chamber is shown in Fig. 1. It is composed of a large aspect ratio cavity of width  $L=15$  mm and height  $h=0.5$  mm with four injection holes located at the corners. In the figure, the 10 mm DNA chip is symbolized by a gray square, located on the lower wall of the cavity. The volume of the chamber is approximately  $110 \mu\text{l}$ . In order to minimize the required quantity of biological material, the height of the fluid layer should be as small as possible. Thus, the present value of  $h=0.5$  mm should be seen as an upper bound, and the use of smaller depths a likely future development.

Fluid flow inside the chamber is induced by imposing a suitable pressure difference between two opposite holes. In the present study, the flow rate is taken to be  $q=20 \mu\text{l s}^{-1}$ . The typical Reynolds number  $R_i$  in the injection pipes of diameter  $d=0.8$  mm is thus

$$R_i = \frac{4q}{\pi d \nu} \approx 30, \quad (2)$$

where we have assumed a kinematic viscosity  $\nu = 10^{-6} \text{ m}^2 \text{ s}^{-1}$  as for pure water. Inside the hybridization chamber, we define the Reynolds number  $R_t$  based on the distance between two opposite holes

$$R_t = \frac{q}{\sqrt{2}L\nu} \approx 0.9. \quad (3)$$

The Reynolds number everywhere in the flow is thus between  $R_t$  and  $R_i$ .

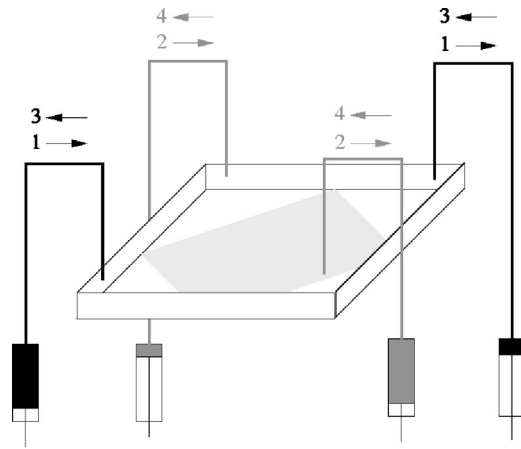


FIG. 2. Alternated injection scheme. Each quarter-period step of the protocol involves two opposite syringes (the black ones in steps 1 and 3, the gray ones in steps 2 and 4), the two other ones being inactive.

Chaotic advection has been successfully applied to other situations of microfluidic flows, such as microchannels, for instance.<sup>11,12</sup> However, the particularity of hybridization chambers is that, due to the very large aspect ratio  $L/h$  of the fluid layer, the flow is almost everywhere of Hele-Shaw type, except near the injection holes. Since the vertical velocity is very weak, the flow can be considered in a first approximation as quasi-two-dimensional. In order to be efficient in this kind of geometry, the chaotic stirring protocol has to be time-dependent: we propose here the simplest such chaotic protocol, based on alternated crossed injections with two pairs of syringes, as shown in Fig. 2. The time-periodic forcing allows horizontal mixing across the cell.

However, since the chip is placed at the bottom of the chamber, one must be sure that DNA molecules visit the whole depth of the fluid layer. The characteristic vertical diffusion time,

$$\tau_h = \frac{h^2}{D} \approx 4 - 40 \text{ min}, \quad (4)$$

is much smaller than the horizontal one, allowing DNA molecules to change horizontal plane in a reasonable time (and, of course, this time would be even less with a thinner fluid layer above the chip). Nonetheless, vertical diffusion is not efficient enough and is augmented by placing the injection pipes vertically, thus creating a large vertical velocity component in the vicinity of the two holes corresponding to the source and the sink at a given time: this allows particles to move from one horizontal plane to another within the hybridization chamber. In consequence, the given flow possesses the properties necessary to good dispersion, both in the horizontal and in the vertical directions, even without allowing for molecular diffusion.

Numerical calculations were performed to determine the fluid particle trajectories generated by the alternating injection system. The computational approach was simplified by considering a steady velocity field during each quarter period. The velocity field was obtained by solution of the Stokes equations in primitive variables using a finite-element method with quadratic approximation of the velocity (the

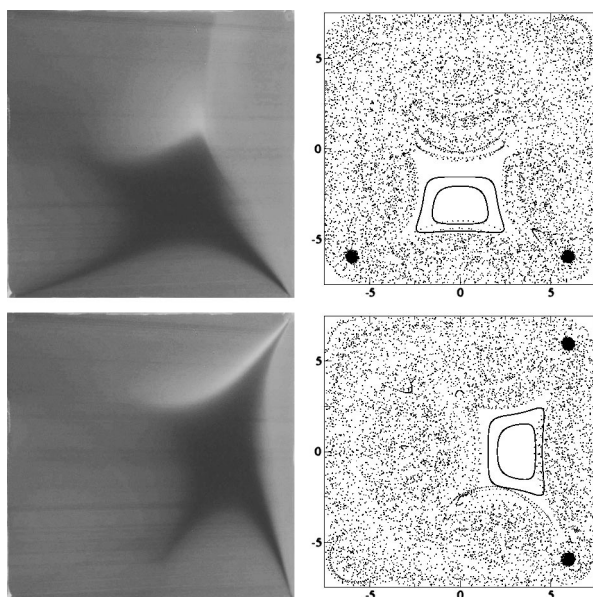


FIG. 3. On the right, the corresponding Poincaré sections from numerical calculations at  $t=6T$  and  $t=6T+T/4$  with  $T=4$  s ( $\underline{T}=420$  s). On the left, the corresponding rhodamine-fluorescence mixing visualization. Note the regular region which moves around with time.

so-called  $P_1-P_2$  or Taylor–Hood element, with linear approximation of the pressure, see Ref. 13). The external syringe system was modeled as four infinitely long straight pipes. The flow was numerically determined out to a distance along the pipe sufficiently large and taken to be Poiseuille flow thereafter. Thanks to flow symmetries, the flow field only need be calculated in a half chamber and completed by symmetry. The resulting steady flow field was then given a  $\pi/2$  rotation each quarter period. Trajectories were determined using standard fourth-order Runge–Kutta integration while Lyapunov exponents (not shown here) were also calculated using a third order scheme to integrate the equations for the Jacobian matrix.<sup>14</sup> Full Navier–Stokes calculations were also performed but with a less accurate scheme ( $P_1-P_{1\text{iso}}P_2$ , see Ref. 13): they essentially recovered the results<sup>15</sup> of the Stokes-flow simulations described here.

Experimental investigations were performed with a 10:1 scaled-up model analogous to Fig. 2. The main goal of this experiment was to check the relevance of the basic ideas and the validity of the numerical approach. Reynolds similarity imposes a flow rate of  $11.24 \text{ ml min}^{-1}$ . Thus, a ratio of  $9.5 \cdot 10^{-3}$  is found between the real period  $T$  and the scaled model period  $\underline{T}$ . A 2 mm-thick horizontal laser sheet was produced using a diverging lens in order to induce rhodamine fluorescence. A drop of highly concentrated solution of rhodamine was injected into one of the external pipes. The alternated injection was then started and top-view photographs recorded every  $T/4$ .

Photographs of the experiment in the case  $\underline{T}=420$  s (corresponding to a period of  $T=4$  s at real scale) are shown in Fig. 3, together with the corresponding Poincaré sections. Very good matching between experiment and numerical simulation is apparent. The reader is cautioned that since the flow is periodic and three-dimensional, the Poincaré section

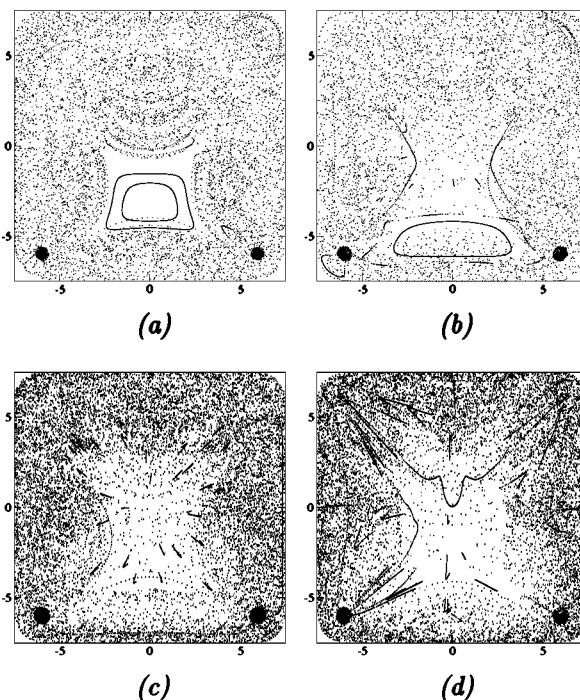


FIG. 4. Top view of three-dimensional Poincaré sections for: (a)  $T=4$  s; (b)  $T=8$  s; (c)  $T=10$  s; (d)  $T=16$  s.

is a set of points in 3D space of which a projection is shown in the figure. It can be seen that, although a large regular region is present (in both the visualizations and numerical simulations), this region moves around with time due to the alternated injection protocol. For the given  $\underline{T}$ , any fixed region of space is visited by chaotic trajectories (and hence by labeled biological material in the real application), even though it may contain a regular region (without any marked particles) for a while. Nonetheless, such regular regions lead to nonuniform DNA concentration across the chip and hence poor analytical performance. In order to avoid this kind of problem, it is important to make sure no regular region exists within the flow.

Top-view Poincaré sections are shown in Fig. 4 for four periods,  $T=4$  s,  $T=8$  s,  $T=10$  s, and  $T=16$  s. Although a regular region was still found for  $T=8$  s, we obtained a completely chaotic flow for  $T \geq 10$  s. The corresponding experiments for these globally chaotic flows are not shown, since the rhodamine drop was fully mixed after only two or three periods. We conclude that mixing is efficient for high enough periods of the flow field. On the other hand, since we are working at a fixed flow rate, increasing the period also increases the volume of fluid inside the syringes: during the quarter period of an injection, the volume of fluid that enters a syringe is  $v=qT/4$ . At the end of a period, two syringes are full, the two others are empty. For the first globally chaotic protocol found ( $T=10$  s), the volume inside a syringe is  $v=50 \mu\text{l}$ ; therefore the minimum quantity of fluid needed overall is almost twice the volume of the chamber, which is still reasonable. Bearing in mind that the smaller the sample of fluid required the better in this particular technology, there is a trade off between mixing efficiency and overall volume. Following this line of thought one can ask what happens if

the depth of the chamber is decreased: since the flow is essentially of the Hele-Shaw type, the problem is largely unchanged if the nondimensional number

$$\frac{qT}{L^2h} \quad (5)$$

is kept constant. Moreover, with smaller  $h$  the effect of molecular diffusion would be increased.

A simple protocol for improving DNA chip hybridization in a large aspect-ratio chamber by chaotic mixing has been proposed in which a time-periodic flow is induced by alternated injections. Numerical simulations have been carried out and validated with experimental visualizations in a scaled-up experiment. The effects on mixing efficiency of varying the flow period have been studied. It is found that the elimination of regular islands, making the flow fully chaotic, occurs for larger values of the period. This corresponds to the injection-extraction of larger volumes of fluid, leading to a trade off with the practical desirability of minimizing the sample volume. Other parameters, such as geometry of the chamber or the position of the injection holes, may also play an important role. There is thus a real need for careful theoretical analysis in order to optimize the mixing process within practical constraints imposed by the technology. Numerical simulations will no doubt have an important role to play in the development process.

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