AN ELECTRONICALLY CONTROLLED MICROFLUIDICS APPROACH TOWARDS ARTIFICIAL CELLS

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Abstract

This work focuses on the application of on-line programmable microfluidic bioprocessing as a complementation vehicle towards the design of artificial cells. The electronically controlled collection, separation and channel transfer of the biomolecules are monitored by a sensitive fluorescence setup. Two different physical effects, electrophoresis and electroosmotic-flow, are used to allow for a detailed micro-control of fluids in micro-reaction environments. A combination of these two basic electronically controlled input reaction chambers makes combinatorial fluidic-networks and indefinitely sustained biochemical or chemical reaction-networks feasible. Experimental data showing the power of this approach is presented. Not only does this processing power pave the way towards the development of artificial cells (using a technology to complement not yet established autonomous metabolic or replication capabilities) but it also opens up new processes for applications of combinatorial chemistry and lab-on-a-chip biotechnology to drug discovery and diagnosis.

Keywords

Reconfigurable hardware, microfluidics, micro-reactor, PDMS, DNA, microelectrode, fluorescence detection

1 Introduction

Integrated microfluidics holds the promise of allowing complex parallel and pipelined processing of combinatorial families of biomolecules without the serial limitations of external robotic pipetting [1]. They allow one to build up dynamic micro-reactors and biochemical networks that are user-programmable, in space and time. Through operational feedback and multiple reconfiguration, they can also be iteratively optimized in an evolutionary process [2]. The development of integrated applications in biotechnology are limited by the necessity of custom hardware and time-consuming development cycles [3, 4, 5, 6]. Reconfigurability opens new possibilities, as one patented strategy allowing researchers to proceed from simple connected micro-reactor elements to dynamically reconfigurable micro-reactor networks [7], shows. These consist of a combinatorial array of processing and switching elements, which can be externally programmed.

Examples of applications are microscale reactors for evolutionary biotechnology, active microfluidics for μTAS and molecular computing in micro-reactors [8, 9, 10]. Artificial containers such as droplets and vesicles in microfluidic environments represent an important area of research in down-scaling combinatorial chemistry but we have proposed that they can also assist to engineer artificial [11, 12] and minimal [13] cells. The important idea in this respect is to use the electronically controlled environment as a substitute for controlled cell functions like metabolism and/or replication, that cannot yet be autonomously regulated. We analyzed the integration of digital microfluidics using immiscible fluids in closed channels, for droplet generation, fusion and transport. Reactors were fabricated in micro-molded PDMS (polydimethylsiloxane) or bonded silicon to glass. We extended previous work [14] to integrate continuous droplet generation and autonomously regulated serial droplet fusion [15]. In the experiments reported here, we incorporated in our microfluidic technology electroosmotic flow [16] (EOF) in addition to electrophoresis and show its feasibility.

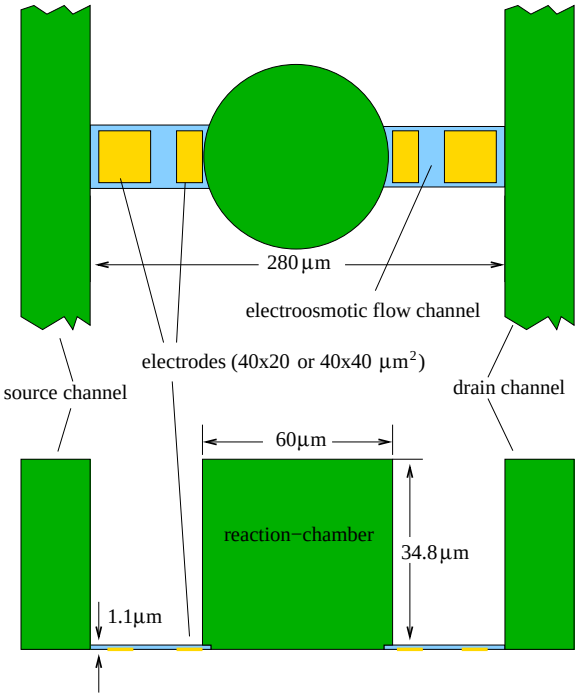

Figure 1: Electronic regulated chamber for an artificial cell connected to chemical supply and drain with two IO-channels of height 1.1μm (measured with profiler P-10 (Fa. KLA-Tencor, USA) and White-Light-Interferometer Wyko NT 1000 Optical Profiler (Veeco Process Metrology)) and regulated by two gold electrodes placed below each I/O. The reaction-chamber, micro-moulded (SU-8 master) in PDMS with size of 34.8μm by 60μm diameter, is effectively decoupled from the hydrodynamic flow of the supply and drain channels, while the programmable electrodes enable adjustable bulk electroosmotic flow (EOF) and electrophoresis. The entire structure can form a basis of a system of programmable microfluidic networks (e. g. Figure 6)

The main unit of our system is an electronically programmable microfluidic chip equipped with micro-electrodes and fluidic channels and chambers, linked to a reconfigurable electronic chip. The micro-electrodes are the actuator components in the microfluidic network, see Figure 1, for the EOF reaction-chamber used in the experiments reported here. Furthermore, the chip contains a standard field-programmable-gate-array (FPGA). The reconfigurable logic device is integrated to control a maximum number of electrodes individually. With this intimate control of biochemical processes a precondition for "live control" comes within reach, that the seamless integration of computer technology and biology occur in both directions simultaneously. This involves both the real time detection and data analysis of the biomolecular system and the active control of biomolecules in a hybrid system [17]. Currently most of our experiments use small DNA oligomers (21 nt, Rh6G–GATGGTCACAGCATGTCTGTS) to test the setup.

Three physical effects are apparent using this technology:

- Electrophoresis
  The electrical field is quite inhomogeneous due to the small electrodes (e.g. 10-40μm) and high field strengths. DNA contains non-aligned permanent dipole moments and a permanent negative charge from the phosphate groups which is partially offset by counter ions from the solvent. Two forces cause electrophoretic molecular transport. The first one is the force of the field on the net charge. This is the same force that drives the ionic constituents of an electrolyte through the solution. The second is the force on the dipole of the molecule: which increases with the divergence of the field. Besides permanent dipole moments DNA also has significant induced dipole moment forces. Note that the strong molecular length dependence of DNA in gels (amplified by chain entanglement) is much less pronounced in free solution. The electrophoretic mobility in free solution in the simplest analysis is the ratio of net charge to hydrodynamic resistance (which follows the Stokes law $F = 6\pi f \eta R$ with an asymmetry correction factor f).

- ElectroOsmotic-Flow (EOF)
  In translation invariant geometry (infinite channels) the Hagen-Poiseuille (H-P) law is valid for a steady-state pressure driven flow of incompressible fluid

$$\triangle p \quad = \quad R_{\mathrm{hyd}} Q \tag{1}$$

  which says that the flow rate Q is proportional to the pressure drop. Electroosmosis, on the other hand, is a non-equilibrium effect, where a liquid is brought to move relative to a charged surface an applied external electric field by acceleration of ions in the solution double layer which screens the surface charge. Surface double-layers are characterized by the ζ-potential. For an ideal EOF at an infinite plate (uniform ζ-potential along the wall, homogeneous E-field, steady-state flow, the Debye layer much thinner than the smallest channel dimension), the electroosmotic velocity is given by

$$u_{eo} = \alpha_{eo} E = \frac{\epsilon \zeta}{\mu} E. \tag{2}$$

  Here α_{eo} is the electroosmotic mobility, ζ is the ζ-potential of the wall, ε and μ are the dielectric constant and dynamic viscosity of the electrolyte, respectively, and E is the applied electric field. Total EOF in a micro-channel is then the sum of individual EOFs

from each wall, each approximated by (2).
In order to obtain stable fluid control into and out of the reaction chambers, Figure 1, it is crucial that pressure driven hydrodynamic flow from the source and the drain channels [3] is suppressed. Flow control can be obtained despite high resistance using electroosmotic flow in many small or a single high aspect ratio channel. To underline this essential mechanism, we first look at an isolated rectangular micro-channel of length L, width W, and height h, with an (infinitely) thin double layer on all walls. The electroosmotic flow rate in it is given by

$$Q_{eo} = u_{eo}hW = \alpha_{eo}V_{\text{eff}}\frac{hW}{L} \propto, \tag{3}$$

where $u_{eo}$is the electroosmotic velocity, α_{eo} the EO mobility, and V_{eff} the electric potential drop inside the channel. Note that this expression implies that electrodes at fixed voltage, placed at either end of a very short electroosmotic channel segment, would produce a very large (diverging) volume flow. Clearly the reason for the problem is that the hydrodynamic resistance of the remaining microfluidic system has not been included. For a high aspect ratio, i.e., $W \gg h$, the hydraulic resistance of the channel is

$$R_{\text{hyd}} = \frac{12\mu L}{h^3 W}\frac{1}{1 - 0.63\frac{h}{W}}, \tag{4}$$

where μ is the dynamic viscosity. The pressure driven flow rate Q_p through the channel is given by (1) with $Q_p \propto h^3$. From (3) and (1) it seems that pressure driven flow will be negligible compared to the EO flow for small values of h, but this only applies for loadless channels.
In the case of electroosmotic channels with a load, e.g. connected to a field free channel segment of hydrodynamic resistance R_L, the lowest order approximation is to use Kirchoffs laws in connection with the low Reynolds number (creeping) flow. Basically, the volume flow for the loadless electroosmotic driven segment is the resting state, and reductions of this flow velocity by the load cause a linear pressure difference across the electroosmotic segment, which can be calculated by (1). Kirchoffs laws imply that the pumped flow velocity satisfies

$$\triangle p_{\text{ext}} + \triangle p_{eo} + \triangle p_L = 0 \tag{5}$$

with the volume flows in the osmotic flow and load channels equated

$$Q_L = \frac{\triangle p_L}{R_L} = Q_{eo} + \frac{\triangle p_{eo}}{R_{eo}} \tag{6}$$

The hydrodynamic resistance is calculated from (4) and the loadless electroosmotic flow from (3). Rearranging, we find

$$Q_L = \frac{R_{eo}Q_{eo} - \triangle p_{\text{ext}}}{R_{eo} + R_L} \tag{7}$$

which for zero external pressure difference reveals a reduction in the loadless osmotic flow rate by a factor of $r = \frac{R_{eo}}{R_{eo}+R_L}$. The underlying additivity assumption is based on a sharp transition from a plug flow profile to a parabolic flow profile at the junction of the driving and load channels.

For our experimental setup, the dimensions of the electroosmotic segments are $W \times L \times h = 40 \times 40 \times 1.1 \mu\text{m}^3$, and the total load resistance is dominated by the electroosmotically non-active part in the horizontal channel, the sink resistors are, compared to this, two orders of magnitude smaller and the source does not even contribute to the total load resistance. This leads to $r = 0.18$, implying a six-fold reduction in the electroosmotic induced velocity. For an overall electroosmotic mobility of $4 \times 10^{-4}\text{cm}^2/\text{Vs}$, an effective voltage of 3.3V, the channel geometry as above, $Q_{eo} = 0.52\mu\text{L/h}$ and without an external pressure difference, the flow in the horizontal channel is given by $Q_L = 0.1\mu\text{L/h}$. In our experiments the non-reversed EOF is directed towards the cathode, indicating negatively charged walls and a positively charged double layer. In PDMS, one common problem is the poorly defined EOF and its dependence on the process used to seal the chip. While it has been reported that an oxidation step is required to produce EOF [18] others claim that no such a step is necessary [19]. The use of multiple materials in a single device, each with a unique ζ-potential, may create non-uniform flows. Currently we cannot distinguish between the EOF created by the SiO_xN_y layer and that created by PDMS walls. The charged site on SiO_2 layers is invariably Si-OH, regardless of the silica type [20] and the presence of silicon nitride also results in predominantly Si-OH charges, with a minority of Si-NH_2 charges shifting the ζ-potential slightly toward positive values, so that the composed layer exhibits roughly similar results. Liu et al. [21] measured the electroosmotic mobility of native and oxidized PDMS/glass devices, both in a range of $4 \cdot 10^{-4}\text{cm}^2/\text{Vs}$ at neutral pH, while, and in contrast to this, Lin et al. [25] investigated the temporary effect of the plasma modified PDMS and found that the ζ-potential decays to zero within two days. Finally, Bianchi et al. [22] presented a model allowing the determination of each individual ζ-potential in composite micro-channels. Here it remains for future work to clearly identify all electro-physical properties.

- Electrolysis
  The applied voltage of 3.3V implies a highly positive reduction potential. Thus it would be possible to perform quite easily an oxidation of the DNA oligomers used in our experiments, since the redox-potentials of DNA are in the range of a few hundred millivolts [23]. Furthermore, the applied potentials should lead to an electrolysis of water of which the redox-potential is +1.23V (O_2/H_2O). Although it is not easy to produce visible electrolysis in the buffer employed in our experiments, this will become an issue if electrodes are driven with DC for more than 10 seconds. Fortunately, it turned out that the experiments with EOF, even when statically driving the electrodes (at constant voltage) for 10 minutes did not show electrolysis. The major reason might be due to the extreme surface-volume ratio (1.1μm height of the channels, see Figure 1). The EOF produced high fluid velocities inside the shallow channels and thus gas-bubbles would be quickly transported away from the electrical field.

2 Overall experimental setup

To distribute the DNA and buffer solutions on the chip a liquid-handling system was connected, see Figure 2. The microfluidic workstation includes high-performance syringe pumps for ultra-low flow rates (MMT). The detection system is based on laser-induced fluorescence and a special optical system as well as a CCD camera for image acquisition. The electrode design was realized with a standard CAD schematic entry system for PCBs (Board Station Mentor

Graphics). The special computer hardware MereGen^{TM} based on reconfigurable electronics [24] analyzes the inner state of the micro-reactor network and realizes the programmability of the system using feed back loops.

The microfluidic device is equipped with gold-electrodes (size typically 20 x 40μm or 40 x 40μm, 96 per module) and a standard field-programmable gate-array (FPGA, SpartanXL XCS20CSP144 Xilinx) that serves as the driver for the electrodes and as the interface to the MereGen board. The electrodes themselves are driven via the standard output drivers of the FPGA meaning a digital 3.3V signal level. With the three states provided (3.3V, 0V and tristate) fast cycling allows us to emulate effectively arbitrary voltage-levels between both extreme values. The configurable logic of the FPGA also serves as a testing-device for the communication buses and built-in self tests.

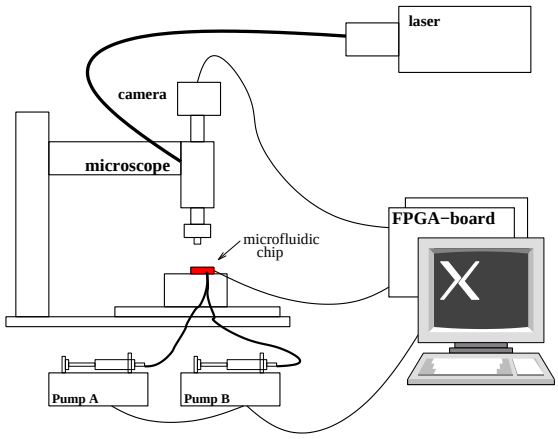

Figure 2: Overall experimental setup. Camera Vosskuehler VDS-QCam 1300QLN (1280*1024, 11 fps), laser argon 514nm, about 100 mW at the chip, Micro-Mechatronic Technologies AG (MMT) pumps (0.2μL/h - 240μL/h with 10μL syringes), custom designed and custom built MereGen^{TM}-FPGA board [24], Linux based VME-bus host-computer (Concurrent Technologies VP 100/01x Pentium III 1.2 Ghz, 512MB), microscope Zeiss Axiovert. The user-friendly control-software is custom-designed.

When molecules are moved, or chemical properties in the fluidic-system changed, fluorescence- or white-light imaging using the camera mounted on the microscope gives the possibility of direct on-line control (see Figure 2). The camera image is preprocessed on the MereGen^{TM} board. Arbitrary regions selected either automatically, via image processing, or manually may be evaluated, statistically processed and subjected to control rules. Depending on the complexity of these calculations, at least the hardest real-time-critical components can be realized in hardware. Introducing low-level control-structures paves the way to connect this work with computer-science on hybrid-systems.

A custom-developed software and operating system, with a user-friendly graphical interface, gives the experimenter an easy access to the underlying hardware. A nice feature of this software is the remote operating capability.

3 Experimental results

Two different solutions in two Hamilton syringe pumps were connected to the two inlets: one solution contained just buffer solution and the other Rhodamine-6G-labeled single stranded DNA with a length of 21 nt. A pump rate chosen between 0 and 2 μL/h was maintained stably in the supply and drain channels. The buffer solution employed was a freshly prepared histidine buffer (50mM, 59μS/cm, pH 7.7) to minimize the electrostatic screening effects (Debye length) [25].

The first task of the experiment was to fill the reaction chamber, see Figure 1, with the labeled DNA. The pump rates were adjusted such that a slow net flow from bottom to top (drain to supply) was maintained if no electrodes were active. At the beginning of the

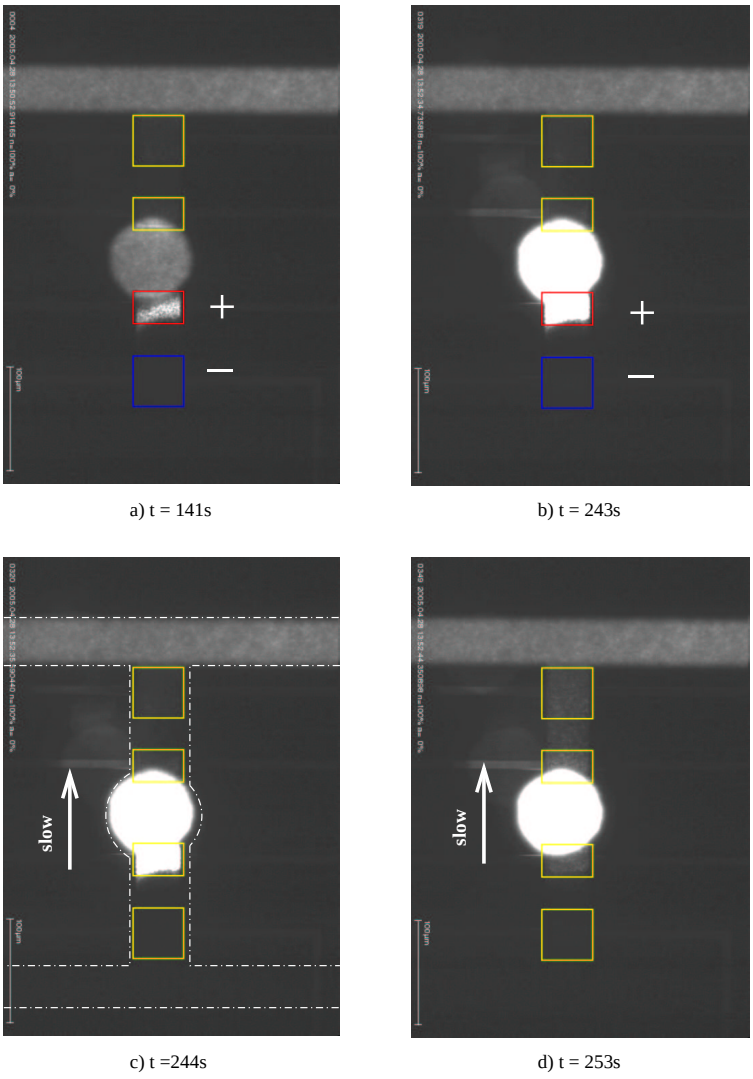

Figure 3: Experimental demonstration of electronically programmable cell filling. Four samples at different times of a filling experiment are shown. The upper horizontal channel (depicted in part c as dashed white lines) is filled with Rh6G $5 \cdot 10^{-6}$ labeled 21mer primer Rev500 batch #33436N in Histidin-buffer 50mM, 59μS/cm, pH 7.7 and excited with laser-light at 514nm with a power of 100mW. At the beginning of the experiment no fluorescence could be observed in the reaction chamber. Two different physical effects are contributing to filling the reaction-chamber: electrophoresis (the positive electrode, 3.3V, a and b) attracts the negatively charged DNA and electroosmotic-flow which drags fluid from the upper supply-channel against the weak background flow from the lower drain-channel upwards. After releasing the electrodes (tristate, high-impedance), material from the reaction-chamber is slowly washed out again. Because of the considerable difference in transport-capacity of the input/output channels and the storage capacity (see Figure 1) of the reaction chamber, this process is quite slow. With an appropriate presetting of the electrode potentials, even this slow dilution can be prevented.

experiment, a faint fluorescence in the DNA-supply ($5 \cdot 10^{-6}$M) channel was visible. The filling procedure startet with setting the second electrode from below to 3.3V, and the bottom electrode to 0V, as shown in Figure 3-a. The two field effects, EOF and electrophoresis, help each other in this case. The negatively charged DNA is attracted from the supply-channel and in addition a net EOF flow is generated which pumps fluid from the supply-channel towards the drain-channel. On the other hand, the electrical field is polarized such that the DNA is repelled from the bottom electrode and attracted by the upper electrode. This results in the observed increase of concentration of DNA, see Figure 3-a-c. Note also the times involved in this filling procedure. Even after more than 200 seconds no electrolysis was observed. Immediately after releasing the electrodes, the slow volumetric flux (from bottom to top of the image) washes the material from the formerly positive electrode back into the reaction chamber (see Figure 3-d). Despite the background flow, the time needed to wash out all the material from the reaction chamber is considerable as a result of the extreme height-ratio between reaction chamber and feeding channels. Further experiments showed (data not shown here) that, with suitably activated electrodes, even this weak outwash process can be drastically reduced and that additionally a reaction in the chamber might be fed continuously.

The second task was to drain the reaction chamber again. Two possible draining directions are available - pumping the material back into the supply-channel (data not shown) which works remarkably fast (typically about 20 seconds are needed to reduce the fluorescence intensity to background level again), and pumping the material against the default flow-pressure into the drain channel, see Figure 4. A time-series of six images is presented. The experiment differed a little from the filling-experiment in that the concentration of DNA in the supply-channel was chosen ten times smaller, $5 \cdot 10^{-7}$M. Everything else remained the same. In this case, the top electrodes are operated, reversing the electroosmotic-flow and expelling the DNA from the reaction-chamber into the drain-channel. After about 50 seconds, the fluorescence intensity in the reaction-chamber was again indistinguishable from the background. As a side effect, the top electrode additionally attracts DNA from the supply channel. This DNA can either be used as a kind of pre-charging for the next reactor filling step or be actively pumped out, after the reaction chamber has been emptied (data not shown). If only the electrodes are deactivated, the high concentration of DNA diffuses along the filling channel in both directions, part of which can be seen in Figure 4-f.

4 From simple single reactors to networks and programmability

The remarkable local control of fluids and concentrations of charged biomolecules shown, and the opportunity to combine this with water-droplets in oil or vesicles in water, immediately raises hopes for strong programmability and combining these features in large networks.

Hybrid-System Programming and Control

Programming in these systems, in the traditional computer-science sense of fixed serial algorithms, is a challenge in several aspects: it has to cope with the continuous versus discrete dichotomy, it involves essentially a stochastic description and it has to cope with reproducibility and error-prone information processing. Even linearization around working-points is difficult in many cases. The first attempts to describe optimal control-strategies with continuous and discrete systems - termed then as Hybrid Automata - were presented by Alur et al. [26] in 1993, as a model and specification language for systems consisting of a discrete program within a continuously changing environment. A major result is that even the very

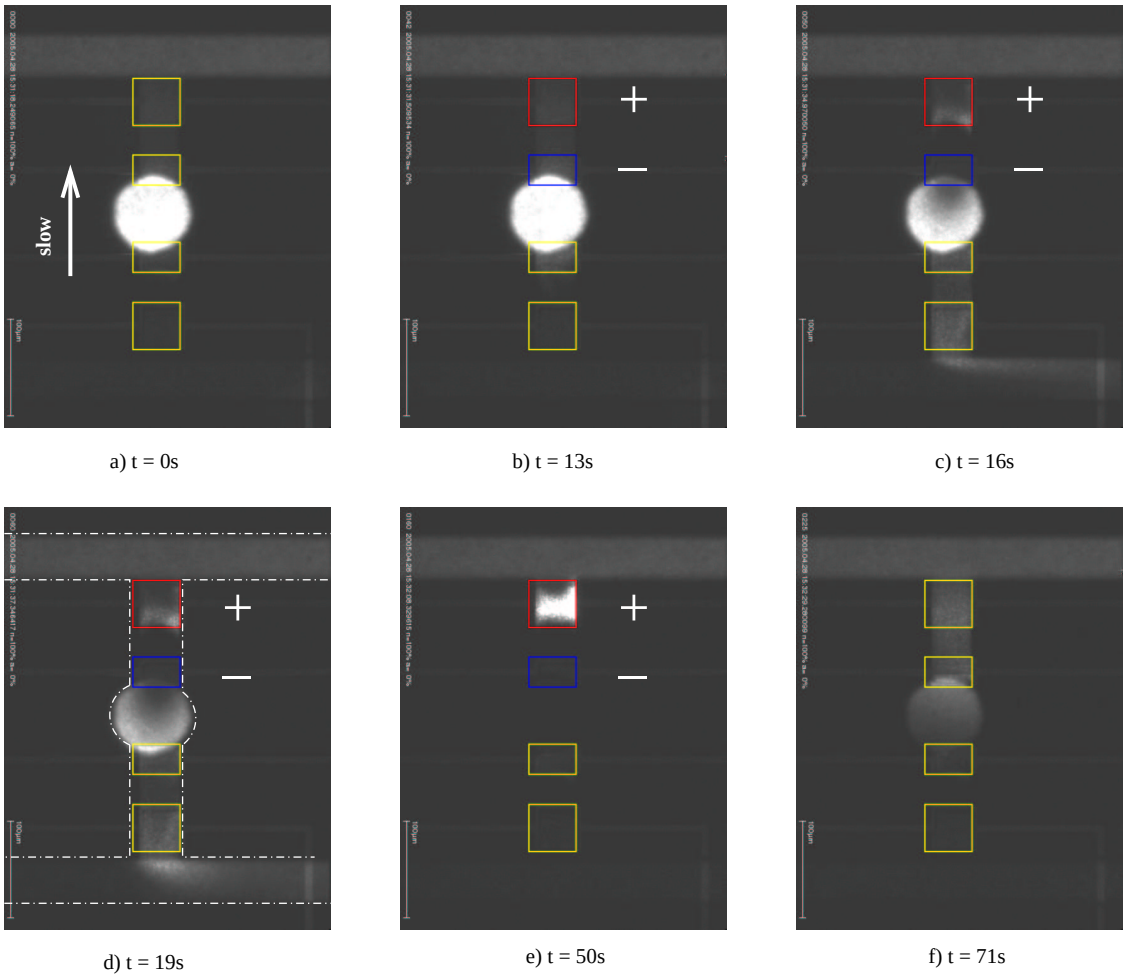

Figure 4: Experimental demonstration of electronically programmable cell draining. Pumping material out of the reaction-chamber is shown in the sequence of six photographs taken at different times. The starting situation is similar to the end-situation shown in Figure 3-d. The concentration of the DNA in the supply-chain has been reduced by a factor of ten ($5 \cdot 10^{-7}$M) before filling the reaction chamber. The two physical forces, electrophoresis and electro-osmosis, empty the reaction-chamber (rapidly considering the huge size of the reaction-chamber in comparison to the shallow input/output-channels) in seconds. A side effect of electrostatic forces is the uptake of DNA-material from the supply-channel (upper channel, sketched as white dashed lines in d). This side effect can be used to create packets of charged material and transport this into a following channel-system. As can be seen in Figure 3, a slow default stream upwards cleans up the reaction chamber after the experiment. Additional suitable electrode control can drastically clean the reaction-chamber (data not shown).

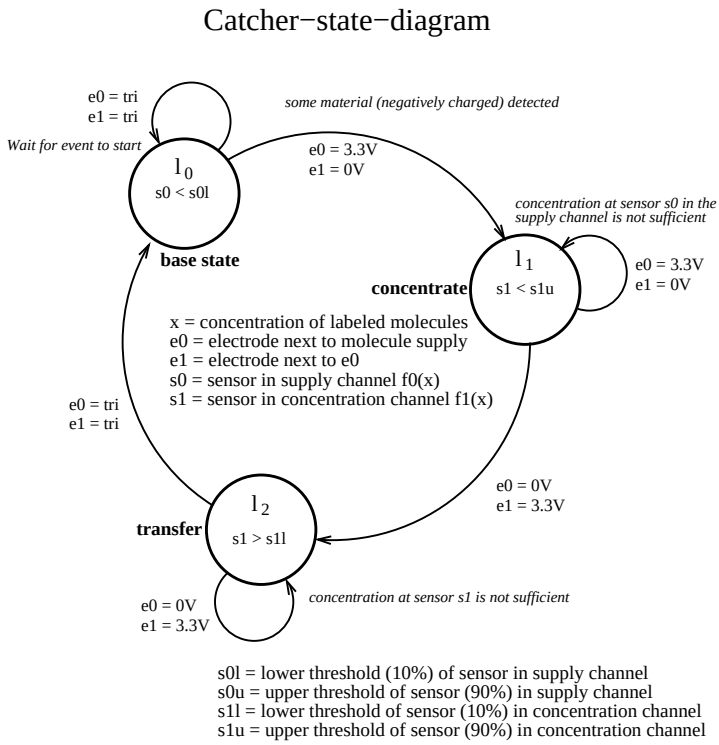

Figure 5: A state-machine diagram (notation used from [26]) of one basic control-module called 'the catcher'. Due to the on-line detection system actively controlled procedures are possible. This one serves for attracting charged material and generating an event to a following stage of the control-network. Such control loops form the link to bridge the gap between microfluidic experimentation and global programming of biochemical processes with software. Noise, detection-problems and other real-world effects can thus be reduced.

simple and restricted linear hybrid automata verification is already undecidable, although three special cases for which verification is possible are presented. In addition to being a hybrid automata, programmability and control of the chip has to cope with stochastic processes because whether molecules are detected or not depends stochastically on their concentration and on surface-properties. This concentration can be either a stochastic continuous variable (10^{14} molecules or more can be seen as continuous reaction-fronts, that may have arbitrary shapes) or discrete when oil-droplets, vesicles or artificial cells are considered. Bujorianu and Lygeros [27] developed a model for general stochastic hybrid systems (GSHS) as a generalization of piecewise-deterministic Markov processes. With the help of dynamic programming, they model GSHS and solve the derived differential equations. Abate et al. [28] describe stochastic approximations of deterministic hybrid systems, again using Markov processes.

Although hybrid-systems research has its origins in classical engineering disciplines, Piazza and Mishra [29] elaborate on questions of stability of hybrid-systems in Systems Biology. Two description-languages in the realm of hybrid-systems seem to be suitable, CHARON [30] and HYSDEL [31], specifically developed as formal specification languages to program and control hybrid-systems. Using the notation of Alur [26], a typical control-element used is shown in Figure 5 and will be discussed here briefly.

Low level regulatory elements

A whole series of low-level control-elements have been devised. The state-machine diagram of one, the catcher, is shown in Figure 5. Two electrodes (actors) and two sensors, suitably placed in the vicinity of the actors are needed. The sensors can produce two signaling events: a drop in intensity of the fluorescent signal below a certain threshold and surpassing intensity above a second threshold. Both thresholds are programmable, even on-line. If the catcher element has captured a sufficient amount of charged material, it signals a following stage (another low-level control element or optionally a more abstract computational entity) that its purpose is finished. The major challenge for incorporating this into control-strategies lies in the non-linear, stochastic and error-prone functional relationship between the concentration of material x and the threshold-parameterized output of sensors s0 and s1. In linear hybrid systems, this relationship is defined as being linear and the proofs of optimal control are based on this assumption. It remains to be experimentally proven that, at least for certain operating conditions, these ill-behaved relationships can be approximated by linear functions or that the current research on optimal control including stability analysis can be extended to these types of functions.

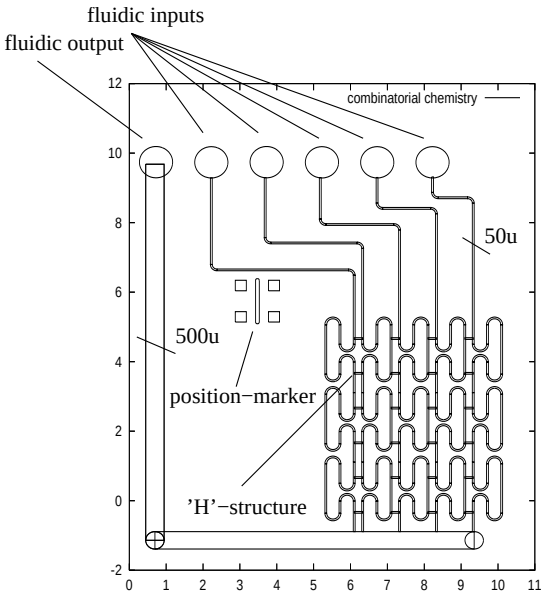

Figure 6: Test-network used to prove the feasibility of combinatorial chemical reactions in microfluidics. The difficult flow-control of this system limited us in the past from doing further research on this topology. However, using the actively controllable reaction-chambers shown in Figure 3 and 4, in a network presented here (graph of a current fluid-design with an n-way fluid-crossbar) combinatorial chemistry in micro-flow-systems will be feasible. Material from each of the five inputs can be brought into contact with each other. The loops shown are used for delay purposes, to allow a synchronized flow behavior. The common output channel not only serves to reduce the number of fluid-connectors but also as a flow-stabilization device (passive regulation).

Combinatorial reaction networks

With these two basic elements, EOF-reaction-chamber and low-level control-elements, the path is open for combining them to create efficient networks. One of the main obstacles in using networks of flow-coupled reaction-chambers, see Figure 6 for a design we tested, is to control the flow. Not only did it prove to be difficult to control the flow in the connecting channel of the H-structures (because of the imperfections which always exist in the fluid-connections and the production process of the imprinted channels in PDMS), it could also be shown that moving DNA packets between the channels do have an effect on the local net flow rates of the fluids in the system (data not shown). With this new strategy, the major flows can now be largely decoupled and independent local cyclic flow-systems can be established and controlled. This latter capability will open up a range of possibilities for evolving artificial cells. More practically at this stage, the role of the specially pulse-free (and expensive) external low-flow-pumps can be reduced to just feeding the system and letting the electrode-systems control the local flow (via EOF).

5 Discussion and conclusion

The aspect of functional complementation of artificial cells that has been investigated here is that of electronically regulated and controllable micro-compartments. To support an artificial cell, chemicals have to be introduced to and removed from these compartments and chemical reactions should be sustained for hours. These long-lasting reactions (the metabolic and replication apparatus in true artificial cells is enzyme-free and thus chemical reactions might take very long times) need to be protected from the outside world and they need to be supplied with the necessary chemicals. Our experimental observation that the transport of charged material worked better for smaller entrance paths, has led to a significant enhancement using EOF. Two physical effects help to alleviate the problems of transport. The strong (at least reciprocal cubic) dependency of the hydrodynamic resistance on channel dimension can be used to decouple channels in microfluidic networks efficiently and drastically reduces fluid-noise in the reaction-chambers. The second effect is the electroosmotic-flow (EOF) that utilizes the ubiquitous ionic-double-layer to produce a net fluid transport under electric fields. Combined, these effects with two-phase systems will further enhance the combinatorial complexity of biochemical reactions which can be regulated in such systems.

The most fascinating potential of this new tool-box is a network of reaction chambers with arbitrary connection-topologies and detailed on-line control. Such systems can benefit from and provide new challenges for the decades of experience and knowledge in computer-science and dynamical system-control. These types of reactor networks can bridge the gap between biochemistry, control-theory, computer-science and "hard" artificial-life research.

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