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Achim Wixforth

Angaben zur Veröffentlichung / Publication details:

Wixforth, Achim. 2005. "Flat fluidics: acoustically driven planar microfluidic devices for biological and chemical applications." In *The 13th International Conference on Solid-State Sensors, Actuators and Microsystems, 2005: Digest of Technical Papers - TRANSDUCERS '05, Seoul, South Korea, 5-9 June 2005*, edited by Bmg-Ki Sohn, Hiroyuki Fujita, and Duk-Dong Lee, 143–46. Piscataway, NJ: IEEE. https://doi.org/10.1109/sensor.2005.1496379.

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FLAT FLUIDICS: ACOUSTICALLY DRIVEN PLANAR MICROFLUIDIC DEVICES FOR BIOLOGICAL AND CHEMICAL APPLICATIONS

Achim Wixforth

Advalytix AG, Eugen-Sänger-Ring 4, 85649 Brunnthal, Germany
and
Chair for Experimental Physics I, University of Augsburg, D-86159, Augsburg, Germany
email: wixforth@advalytix.de

ABSTRACT

A novel technology incorporates pumps and valves without moving parts on a chip surface. Using these building blocks that can be combined in a plug-and-play manner, biochemical reactions on the chip are controlled electronically.

Keywords: Surface Acoustic Waves, microfluidics, biochip

The knowledge of the biochemical interior of living cells steadily increases and researchers dig deeper and deeper into the biomolecular world. Now the human genome is sequenced, scientist hope to find novel drug targets once the code is cracked.

Analytical techniques like gene expression analysis or cell assays have become standard tools, used in large scale screening for new drugs. The very same technologies are the driving force behind the need for miniaturization of the laboratories, as parallel screening requires smallest amounts of sample for the single experiments. The resources of many of the precious ingredients are either very limited or prohibitivly expensive.

Following the evolution from testtubes to micro titerplates the move to biochips seems all but inevitable. The number of individual wells is steadily increasing, reducing the sample volume. It seems quite natural that the MTPs will be followed by even smaller devices that should also be smarter than just providing a container. However, continuing miniaturization requires a large step: Crossing the border between the „macro" and the „micro". Here, new physical effects, which are negligible in the world of the daily life become more and more dominant. Surface tension for example beats gravity.

Squeezed into micron size tubes, water suddenly appears as viscous as honey, making controlled pumping a difficult task. Mixing of two fluids in the micro world resembles the kneading of a dough. The engineers have tried to beat the laws of nature in the microfluidics world for quite a while. However, these laws are very resistant and many nano plumbing problems remain unsolved so far.

We have a different strategy: If you can't beat them – join them! The dominant forces in the nano world aught to be friend, not foe. Self organization and self assembly are the key concepts for this new generation of microfluidic devices. Small amounts of liquid kept in virtual beakers are transported across the surface of a chip, driven by surface acoustic waves. It's the surface tension trapping the reagents in tiny droplets forming such beakers.

FLAT FLUIDICS

The spontaneous formation of droplets for small amounts of fluid is driven by surface tension effects on the other hand their size and shape is governed by the chemical and physical properties of the surface on which they form. Spider webs on a summer morning are a perfect example: Each string is covered with a series of dew droplets, each string providing the rules for the formation of well defined sizes. In terms of the design of a microfluidic device, it is the task of the scientist to deliberately engineer the surface properties of a chip.

Employing tricks like self-assembling monolayers of special chemicals or creating a specific nano structure on the surface, we are able to lay down fluidic tracks, very much like printing circuits on an electronics board. This way, specific regions of the chip surface can be made hydrophillic or hydrophobic. Lithographic techniques known from semiconductor fabrication can be employed to give these tracks most any desired shape. Design libraries can be created: building blocks for complex two-dimensional fluidic networks. Small amounts of reagents are confined to these tracks, without the need for mechanical barriers used in conventional microfluidics. Moreover, such two-dimensional wetting structures are very easy to manufacture at low cost – a key factor for commercialization.

Conventional pumps, of course, are useless to actuate the liquid in the flatland of our two-dimensional laboratories. We came up with a completely different idea: the integration of nanopumps on the very same chip substrate. Using a piezo-electric chip material we generate so-called surface acoustic waves (SAW) by applying radio-frequency signals to specially designed electrodes (interdigital transducers, IDT).

Such surface waves propagate across the substrate just like earthquakes do. SAW-devices have been around for years in communication circuitry – every cell phone has

filters using the effect. An electrical signal fed into the IDT transducers on the chip surface is converted into a deformation of the crystal underneath. Given the right frequency of the signal, a mechanical wave is launched across the chip. Having wavelengths of a few microns and amplitudes of about only a nanometer, the forces within the nano-earthquake are sufficient to have a macroscopic effect. Any piece of matter at the surface along their way experiences their vibrating force: viscous materials like liquids absorb a lot of their energy. Low power SAW induces an internal streaming inside reagent volumes, SAW of larger amplitudes moves the droplet as a whole [1].

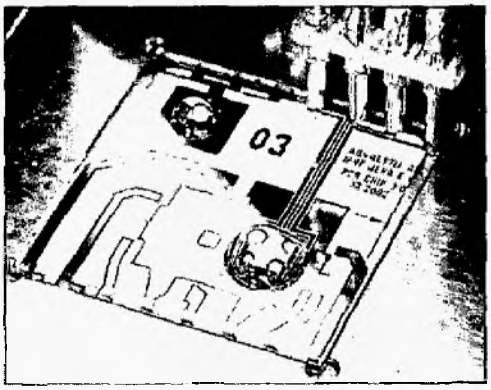

Fig. 1: A complete Polymerase Chain Reaction (PCR) chip based on acoustically mediated liquid actuation. The PCR solution has been dyed red to provide a better contrast to the oil used as a cover. The spring contacts on the chip's upper side lead to the temperature sensor and the resistive heater. The PCR reaction center is located inside the heating coil and covered by four drops of PCR solution. The window to the left of the reaction spot (light square) was used for spotting the hybridization oligos. Two SAW transducers can be seen both on the left and on the right hand side of the PCR reaction center.

The droplets are manipulated on the chip's surface where certain areas have been chemically modified to define hydrophilic tracks. These tracks ensure that the drops will follow a well defined trajectory along the propagation direction of the SAW preventing any deviation to the sides. The surroundings of these tracks are hydrophobic such that the drops have contact angles at their perimeter of about 60° to 120°. The acoustically mediated actuation of the drops can for instance be used to bring sample and reagent to reaction by merging a drop of sample solution with a drop of reagent. The same ultrasonic technique was also employed to agitate the drops, and hence to accelerate (bio-) chemical reactions on the chip [2].

Our nano pumps based on SAW technology have no moving parts. They can be manufactured in a simple lithographic process, very much like the metallization of computer chips. The same technology can be used to define the fl uidic tracks. Fig. 2 depicts the basic mechanism behind the SAW induced streaming of liquid on the surface of a chip.

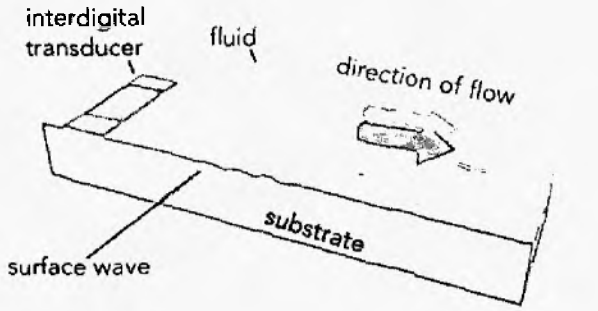

Fig. 2: Pumps without moving parts – a high frequency signal applied to the transducer electrode (left) on a piezoelectric substrate is converted into a surface acoustic wave. The interaction between this nanoquake and a fluid film on the chip surface actuation the liquid.

Making use of the SAW induced acoustic streaming in such small volumes, SAW enables us to build the probably world's smallest mixers. As has been pointed out above, at low power levels, the SAW is converted into an internal streaming in a fluid located on the chip surface. This streaming induces a very efficient mixing and stirring within the small volume. Especially, if the frequency of the SAW is changed during this process, different streaming patterns are induced and superimposed, leading to quasi- chaotic mixing patterns. This is a true advantage in the field of micro-fluidics: Mixing and stirring, as simple as it sounds, is one of the major challenges. Dozens of different techniques have been designed to overcome the rules of nature in the micro world. However, most of the mixing of low volume fluids is still left to diffusion – a rather slow process, as anybody knows who has ever waited for sugar to dissolve in his or her coffee for want of a spoon. In fig. 3, we show a time series of such a nano stirrer at work: A fluorescent dye on the surface of a chip is covered with a small water droplet. Under the influence of the SAW the dye quickly dissolves illuminating the droplet homogeneously – orders of magnitude faster than by diffusion alone [3].

At higher power levels, the internal acoustic streaming in a droplet or any other closed fluid volume turns over to a movement of the droplet as a whole. An optimized SAW profile together with an appropriate timing of SAW pulses pushes the droplet along predetermined pathways along the chip surface. As the SAW is excited electrically by charging the IDTs with an RF signal, many different droplets can be manipulated simultaneously on the same chip. Of course, this requires a set of different transducers for a given SAW direction each. Apart from acting as nanopumps, the presence of different IDTs on the chip is very useful for the electrical detection of a droplets position on the chip. For

this purpose, two opposing IDT are operated as a SAW delay line. Once a droplet, being pushed by a SAW, enters the acoustic path of this delay line, the transmission of this second SAW is dramatically reduced as opposed to the unloaded case. We use this signal reduction as an indication of the presence of a small droplet at the intersecting point of the two SAW beams. This way, a computer program can operate the chip autonomously in a feed back manner.

Fig. 4 shows four snapshot of three droplets being transported and processed by nano-quake on a chip surface. Note that by employing planar technology, complex additional features like sensors, heaters etc. can be fabricated on the same chip surface. The volume of these droplets is only about 100 nanoliters – very small test tubes, indeed.

Fig. 3: Nanoliter mixing: dissolving of a fluorescent dye deposited on the chip surface. Acoustically induced mixing of the fluid leads to a homogenous blend of water and dye.

APPLICATIONS

First applications of the SAW driven microfluidics are the „ArrayBooster", and the „SlideBooster" [4] , both hybridization stations for a variety of different applications, ranging from DNA- and protein microarrays to immuno histochemistry applications. The efficient agitation of the sample fluid during the incubation significantly accelerates the hybridization [2]. Moreover, the homogeneity of the fluorescence signals across individual spots is greatly enhanced, spot-to-spot variations are reduced and the signal-to-noise-ratio increases. The instruments allows for minimal sample volumes down to only 10 microliters.

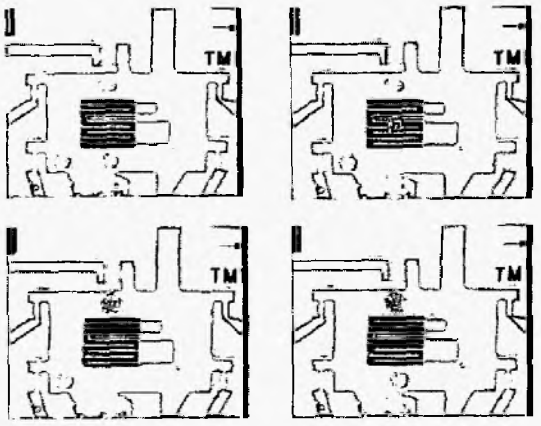

Fig. 4: Subsequent snapshots of a programmable biochip in action. Three different chemicals of about 100 nl volume each are manipulated along the surface of the substrate. Electronically controlled two-dimensional movement of the droplets is used to merge and mix the separate small volumes and to induce a chemical reaction (color change).

To prevent evaportaion of small droplets at elevated temperatures, we have developed a technique to cover the aquaeous solution by a little oil droplet [5]. This way, the we generate a fluidic test tube, holding the precious sample even at higher temperatures like needed in a PCR process. For the PCR chip assays (like the one shown in Fig.1), we employed the SAW technology to dissolve the primers and all other PCR reagents which had been dried on the chip prior to the experiment. We have found no deterioraton of the amplification efficiency owing to the PCR reagents being dried on the substrate rather than added as a liquid. After the amplification we used SAW to move the amplicon to an hybridization window on the chip (cf. Fig.1). During this transfer we took care that the oil continued to cover the PCR solution completely so that the subsequent hybridization could also be perfomed underneath the same oil cover. The continous coverage with oil was achieved by slowly increasing the RF voltage until the PCR solution had reached its final destination at the hybridization window. We then hybridized the PCR products to the oligonucleotide probes spotted inside the hybridization window. In Fig. 5 we show the fluorescence signal of PCR products amplified on the chip hybridized to their complimentary oligonucleotide probes (duplicate on the right) and to negative controls (duplicate on the left). By this on-chip detection via hybridization we also could verify the specificity of the PCR products.

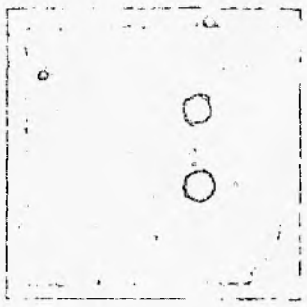

Fig. 5: Microarray hybridization on the chip. Two duplicate spots were used to perform a positive (right), and a negative (left) control experiment. During hybridization, the sample was gently agitated, again using the surface acoustic wave technology.

As a next step we investigated PCR reactions using different dried-on primers covered by only one drop of oil as depicted in Fig.1 (multispot PCR). Our intention was to check for any cross contamination due to the spotting of the PCR primers onto the chip followed by a transfer of primers from one spot to another during storage. We considered an additional source of contamination to be spotted primers leaking into adjacent PCR solutions during the cycling as the strong temperature gradients may lead to substantial convection inside the drop which in turn will distort the drops of PCR solution.

To examine the cross contamination issue we amplified the same sample with three different primer sets under one drop of oil. The primers were chosen such that they would result in PCR products of different lengths. As a negative control we included one spot without any primers.

Fig. 6: Gel electrophoresis for a multispot PCR on the acoustically driven biochip. Lanes 1, 2, and 3 represent the results for different primers, lane x shows a negative control experiment within the same "virtual" reaction tube.

The results were analyzed using gel electrophoresis as can be seen in Fig. 4. There are three different bands for the spots 1,2, and 3 whereas the negative control (x) shows no signal. We conclude from this result that it is indeed possible to have the primers dried on the chip and perform PCR reactions underneath a single drop of oil acting as a "virtual" test tube without risking any cross contamination.

CONCLUSIONS

In summary, we have described an novel, unconventional method to manipulate small amounts of fluid on the planar surface of an electrically programmable chip. Iinstead of using closed tubes, trenches, or channels to confine and guide the fluids, we employ surface functionalization to creat "virtual" containers and tracks for the liquid on the planar chip surface. Actuation of the liquid is achieved by the interaction of the fluid with surface acoustic waves that act as "conveyor belts" without moving parts. Apart from many other applications, we were able to show that hybridization assays largely benefit from the controlled acoustic agitation and internal streaming of the sample fluids. At larger amplitudes, the SAW is also able to actuate a droplet as a whole. This enables us to device quite complex assasy which then result in a predetermined woorkflow on a programmable biochip.

ACKNOWLEDGMENT

This work has been sposored in part by the German ministry of Research and Technology (BMBF), in part by the Bayerische Forschungsstiftung under the program FORNANO, and in part by the German Science Foundation (DFG) under the priority program SFB 486.

Finally, I thank all my collegues with Advalytix, and EP1 at the University of Augsburg for their fabulous work, enthusiasm, and many fruitful discussions.

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