Acoustically driven planar microfluidics

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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, scientists hope to find novel drug targets once the code is cracked. Analytical techniques such as gene expression analysis and cell assays have become standard tools, used in large scale screening for new drugs. The very same technologies are the driving force behind miniaturization of the laboratories, as parallel screening requires smallest possible amounts of samples for single experiments. Many of the precious ingredients are either very limited in availability or prohibitively expensive.

In this article, we wish to report a novel way of tackling the need to scale chemical and biological laboratories down to the size of a thumbnail. We describe a technique which uses virtual beakers and channels to confine smallest possible amounts of liquids to the plane surface of a microchip, and tiny earthquakes (surface acoustic waves, SAW) on this very chip to act as electrically addressable and programmable nanopumps. The combination of the two can be viewed as a step towards the realization of a programmable microfluidic processor.

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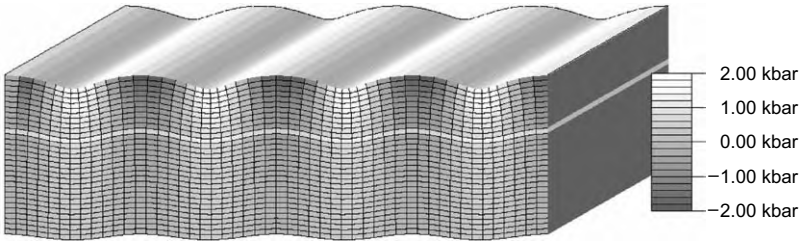

Fig. 1. A sketch of a surface acoustic wave propagating on a piezoelectric substrate. Typical wavelengths are in the micrometer range, typical amplitudes less than a nanometer.

Surface acoustic waves were first described in combination with earthquakes [1]. Meanwhile, reduced to the significantly smaller nanoscale, they found their way into much friendlier fields: SAW devices are widely used for RF signal processing and filter applications and became the basis of a huge industry in mobile communication. SAW devices have been around for years in communication circuitry—every cell phone has filters using the effect. An electrical signal fed into so-called transducers on the surface of a piezoelectric chip is converted into a deformation of the crystal underneath. Given the right frequency of the signal, a mechanical wave is launched across the chip. In Fig. 1, we sketch a snapshot of a SAW propagating on a solid. The SAW is characterized by subsequent regions of compressed and expanded material as indicated in the grayscale.

In the recent past, SAW have also been used to act in a completely different way than for filtering and signal processing just by converting electrical signals into mechanical vibrations and vice versa. Excited on piezoelectric substrates, they are accompanied by large electric fields. Those electric fields are traveling at the speed of sound of the substrate (approx. 3000 m/s), having the same spatial and temporal periodicity as their mechanical companions. Charges at or close to the surface are coupling to these electric fields, and currents are induced within a conducting layer. Nearly twenty years ago we thus introduced SAW to study the dynamic conductivity $\sigma(\omega, k)$ of low dimensional electron systems in high magnetic fields and at low temperatures. It turns out that the interaction between a SAW and the mobile charges in a semiconductor is strongest for very low sheet conductivities as observed, e.g., in the regime of the quantum Hall effect [2]. However, SAW can be used not only to probe the properties of quantum systems, but also to deliberately alter some of them, as SAW represent a spatially modulated strain and stress field accompanied by strong electric fields in a solid, and propagating at the speed of sound. Such an interaction between SAW and the optical properties of a semiconductor quantum well led us to the discovery that photogenerated electron–hole pairs in a semiconductor quantum well can be spatially separated under the influence of a SAW mediated electric field. This, in turn, has an enormous impact on the photoluminescence (PL) of the semiconductor. We were able to show that the PL not only is quenched under the influence of a SAW, but also can be re-established at a remote location on the sample and after a certain delay time [3]. Further studies include the acoustic charge transport and the creation of dynamically induced electron wires [4], as well as the study of nonlinear acoustic interaction with low dimensional electron systems in semiconductors [5].

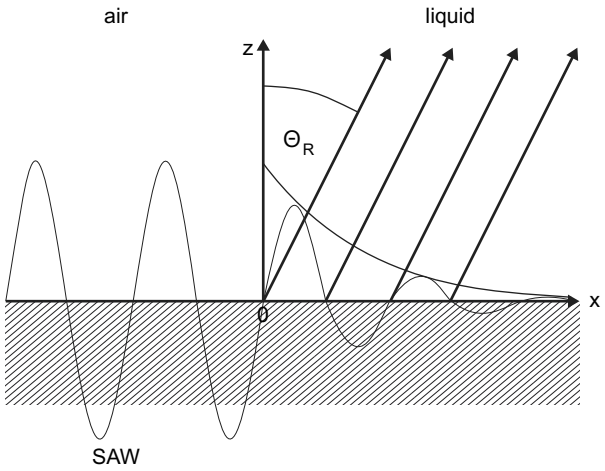

Fig. 2. A schematic illustration of the interaction between a SAW and a liquid at the surface of the SAW substrate. The SAW is propagating from left to right, and at $x = 0$ hits the liquid. A longitudinal sound wave is radiated into the fluid under a refraction angle Θ_R.

However, the piezoelectric effect is usually only a small contribution to the elastic properties of a solid: most of the energy propagating in a SAW (usually more than 95%) is of mechanical nature [6]. Hence, not only electrical interactions as described above, but also mechanical interactions are a possible field for experimental investigations. Having wavelengths of a few microns and amplitudes of about only a nanometer, however, the forces and electric fields within the nanoquake are sufficient to have a macroscopic effect. Any piece of matter at the surface along the path of a SAW experiences their vibrating force; viscous materials such as liquids absorb a lot of their energy. It turns out that the interaction between a SAW and a liquid on top of the substrate surface induces an internal streaming, and, as we will point out below, at large SAW amplitudes this can even lead to a movement of the liquid as a whole.

The origin of such an acoustically induced internal streaming is depicted schematically in Fig. 2. A SAW is propagating from left to right along the x-axis. At $x = 0$, it reaches the boundary of a liquid at the surface of the substrate. A SAW with non-vanishing amplitude in the z-direction, i.e. normal to the surface of the substrate, is then strongly absorbed by the fluid, as indicated by the decaying amplitude for positive x values. Moreover, it creates a small but finite pressure difference $2\Delta p$ in the fluid between the ridges and the wells of the wave, which transforms into a small but finite difference $2\Delta\rho$ in the liquid density. Both quantities then spatially and temporally oscillate around their respective equilibrium values, p_0 and ρ_0, respectively. The pressure difference directly above the surface of the substrate leads to the excitation of a longitudinal sound wave into the liquid. As the sound velocities for the liquid and the solid substrate are in general not equal, this wave is launched under a diffraction angle Θ_R, given by

$$\Theta_R = \arcsin\left(\frac{v_S}{v_f}\right). \tag{1}$$

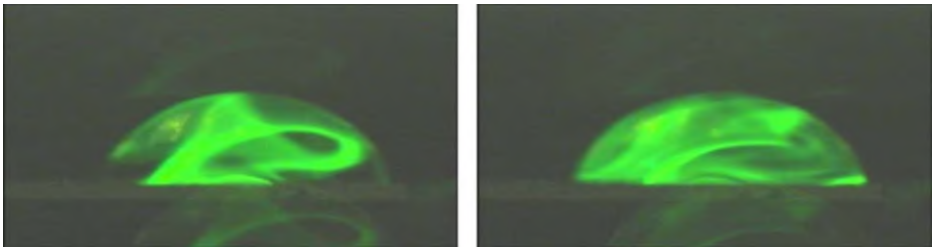

Fig. 3. SAW induced internal streaming in a small water droplet (side view, approx. 50 nl). A dried fluorescent dye on the surface of the chip is dissolved by SAW agitation, and rapidly fills the whole droplet volume.

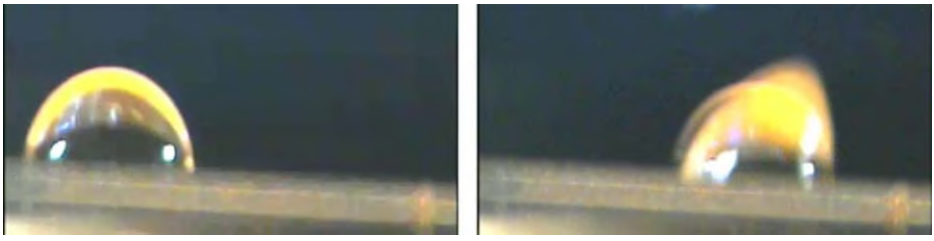

Fig. 4. A side view of a small droplet (ca. 50 nl) on the surface of a piezoelectric substrate. Left: the droplet at rest; note the wetting angle of about 90°, which has been obtained by hydrophobic treatment of the surface. Right: the droplet being 'hit' by a SAW impinging from the left. The acoustic radiation strongly deforms the droplet shape. This leads to a momentary asymmetry of the wetting angles of the droplet.

Here, v_S and v_f denote the sound velocities of the substrate and the fluid, respectively. In addition, the SAW is responsible for the build-up of an acoustic radiation pressure [4]

$$P_S = \rho_0 v_S^2 \left(\frac{\Delta\rho}{\rho_0} \right)^2 \tag{2}$$

in the direction of the sound propagation in the fluid. This leads to an internal streaming in a closed volume such as a droplet, as the boundary of the droplet reflects the actuated fluid back to the source. Such internal SAW driven streaming in a small droplet can be nicely visualized by dissolving a dried fluorescent dye under the influence of a SAW. In Fig. 3, we show two snapshots of such a fluorescence experiment, taken approximately one second apart from each other.

For larger SAW amplitudes, the acoustic radiation pressure even deforms the droplet surface at the opposite side of the sound entrance. This can be seen in Fig. 4, where we show a droplet under the influence of a quite intense SAW, impinging from the left. As can be seen from the figure, the acoustic radiation pressure in this case is so high that it strongly deforms the droplet. At the same time, the wetting angles to the left and the right of the droplet (i.e. 'luff and lee' of the SAW) are modified with respect to the equilibrium values. This acoustically driven deformation of the droplet is the main reason for the droplet actuation in our case.

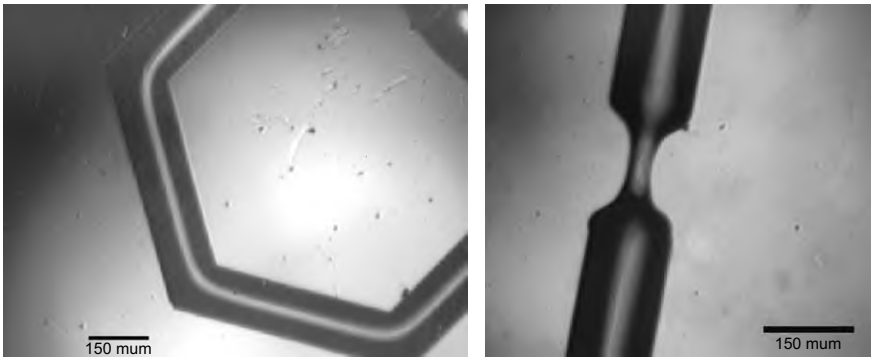

Fig. 5. Photolithographically defined surface modification to modulate the wettability. In this way, 'virtual' fluidic tracks are created to confine the smallest possible amounts of liquid to predetermined geometries or to guide a SAW driven droplet along a predetermined path on the chip surface.

The next step towards an application of SAW in microfluidics in general is to create 'flatland' analogs to channels, tubes, reservoirs, mixing chambers and similar building blocks usually employed to guide, contain or process liquids in a fluidic network.

By a chemical modification of parts of the chip surface we are able to create patterns of preferred wettability (hydrophilic regions), being separated by regions of surface chemistry where fluids are repelled (hydrophobic areas). Employing lithographic techniques borrowed from semiconductor microelectronics, we thus can create completely flat, two-dimensional fluidic networks, where liquids are confined to virtual tracks, reservoirs and reaction chambers by surface tension alone.

In Fig. 5, we depict some of such self-assembled virtual potential wells for fluids on a surface. Photolithographic techniques have been employed to create 'containers' for smallest possible amounts of liquid, having predetermined shapes such as a hexagon (left) or a 'tube' with a narrowing (acting as a pressure driven valve, by the way). Given this surface functionalization, either closed fluid volumes or single droplets may be acoustically guided along predetermined pathways along the surface of the chip.

Given such chemically defined virtual tracks and the SAW based actuation, we can thus move several droplets of different fluids (or generally different reagents) independently in any desired direction. In Fig. 6, we give a series of snapshots of one of our fluidic chips, where three droplets are actuated using SAW agitation. Depending on the actual layout of the chip, the droplets can be split into smaller ones, and they can be merged, mixed and processed in almost any fashion. As the SAW nanopumps are electrically addressable, a complete sequence of different steps of a chemical 'experiment' or a biological assay can be computer controlled. Moreover, the simplicity of the fabrication process of our 'programmable bioprocessors' makes them serious candidates for providing truly miniaturized laboratories on a chip [7].

First applications of the SAW based fluidic actuation already exist and are currently commercialized: the ability of SAW to efficiently stir and mix smallest possible amounts of fluids is employed to enhance the results of biological hybridization assays. During hybridization, immobilized DNA fragments or oligonucleotides, which are spotted on a

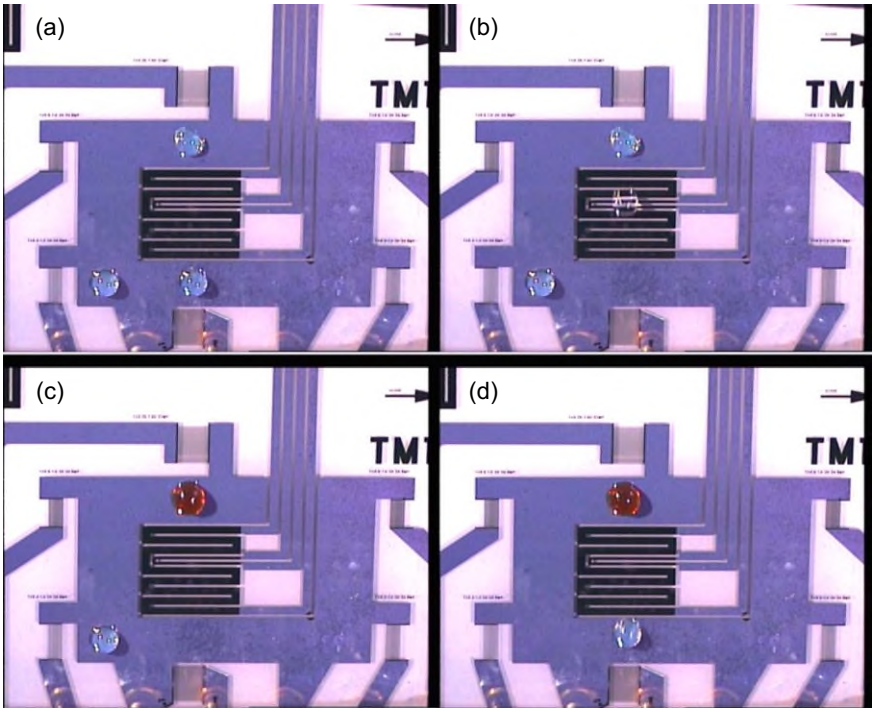

Fig. 6. A SAW driven microfluidic processor. Three droplets (approx. 100 nl each) are moved in a 'remotely controlled' manner and independently by the nanopumps. (a) through (d) represent a series of subsequently taken snapshots, to visualize the movement and the 'nanochemical reactions' occurring when the droplets are merged and mixed by the action of the surface wave. The chip not only contains the nanopumps and the fluidic environment but in the center additional 'real estate' such as sensors and heaters.

microarray, are flooded by an unknown sample fluid, containing other oligonucleotides. Once hybridization occurs, fluorescence markers at the sample molecules accumulate at a specific spot. This fluorescent signal can then be detected and act as a measure for the hybridization efficiency, in other words, the degree of matching between sample and target molecule.

Usually, such microarray hybridizations are performed in a thin capillary fluid film (thickness approx. 50 μm), spread across the area of a conventional microscope slide (7.5 cm × 2.5 cm). Here, the narrowness of the film again leads to a complete suppression of turbulent flow in the film; diffusion is the only driving force to move a sample molecule towards a target spot.

In Table 1, for instance, we have calculated the diffusion time needed to cover a certain distance in such a thin liquid film. To cover a distance of only 1 mm, a 100 nm long DNA fragment already needs approximately 30 h. This diffusion limit together with the then unavoidable depletion effects leads to a very slow hybridization process for microarrays. Obviously, mixing and stirring the fluid in the narrow slit would improve this process, as the diffusion limit can be overcome in this case. Although even SAW mediated agitation in

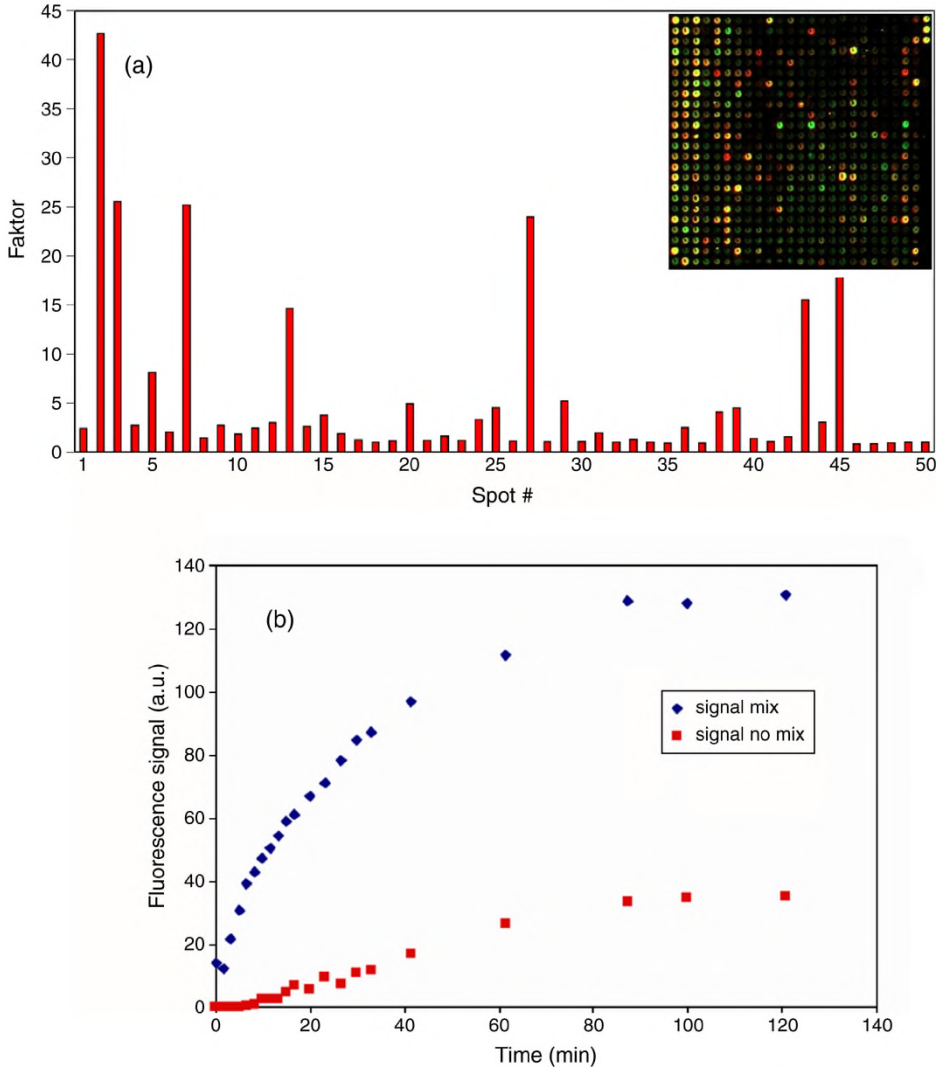

Fig. 7. The result of a fluorescence labeled microarray experiment (a rat chip with 50 oligos, three subarrays, two replicas each, overnight hybridization at 42 °C). In (a), we show the intensity enhancement for different spots on the microarray for the agitated sample fluid as compared to the diffusion-limited case. The inset shows a typical microarray. In (b) we depict the temporal evolution of the fluorescence intensity in such an experiment.

a thin liquid film is rather slow as compared to a free fluid surface, the diffusion limit can be definitely overcome as we show in Fig. 7.

Here, we depict the result of the fluorescence analysis of a typical microarray assay for diffusion only, and the mixed case. Not only do we observe a quite dramatic increase of the signal intensity in the latter case, but also the homogeneity of the single spots on the microarray is much better for the agitated sample [8]. Another important issue that

Table 1
Diffusion times for different diffusion lengths and three different particle sizes, calculated for $T = 20\,^{\circ}\mathrm{C}$. A DNA segment of only 100 nm length needs about 30 h to diffuse over a distance of only 1 mm

Diffusion length (μm)	Potassium ion (0.2 nm)	Oligonucleotide (6 nm)	PCR product (100 nm)
1	0.2 ms	6 ms	100 ms
10	20 ms	600 ms	10 s
100	2 s	60 s	20 min
1000	200 s	100 min	30 h

is associated with microagitation of microarray hybridization assays is the fact that by overcoming the diffusion limit, it is now possible to actually monitor the temporal evolution of the hybridization process. This gives researchers an additional channel of information for further improving the results of such macromolecular experiments.

In summary, we have described a novel and unconventional method for microfluidic fluid handling at very small sample volumes. Surface acoustic waves on a piezoelectric substrate strongly interact with a liquid at the surface of this substrate, which leads to the build-up of an acoustic radiation pressure in the fluid. This pressure is basically the origin for the SAW mediated internal streaming in the fluid as well as for the actuation of small droplets as a whole. Laterally patterned surface modification in addition enables the creation of fluidic tracks, containers or functional elements for a planar microfluidic network. Programmable actuation of different small droplets together with a wealth of possible fluidic functional blocks represents a step towards the realization of a programmable fluidic microprocessor.

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