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Flat fluidics: a new route toward programmable biochips

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ABSTRACT

The miniaturization of chemical and biological processes has made enormous progress driven mainly by genomics and proteomics. Microfluidics is the core technology to realize miniaturized laboratories with feature sizes on a submillimeter scale. Here, we report on a novel microfluidic technology which allows biochips to be programmed so that different biological assays can be performed with only one chip layout. Interdigital transducers integrated on piezoelectric substrates excite surface acoustic waves (SAW) which drive reagents on the surface of the biochip. The reagents can be placed on any desired spot on the chip's surface, they can be merged, split and brought to reaction. SAW technology can also be used to efficiently agitate small volumes of liquids accelerating diffusion limited reactions considerably.

KEYWORDS: biochip, microfluidics, DNA microrarray, surface acoustic waves, acoustic streaming, laminar flow

1. INTRODUCTION

Microfluidic systems miniaturize chemical and biological processes on a submillimeter scale. Reducing the dimensions of macroscopic biological or chemical laboratories is advantageous as the small scale allows for the integration of various processes on one chip analogous to integrated microelectronic circuitry. Also, the required reagent volumes are reduced which saves material costs and allows the reactions to be carried out at high sample concentrations. These high concentrations drive the reactions towards the products' side and accelerate the kinetics. Finally, miniaturization results in enhanced precision by providing homogenous reaction conditions.

Several approaches to realize microfluidic systems have been reported in the literature. In most cases the reagents are moved in channels or capillaries with typical diameters ranging from 50μm to 500μm. These channels can be fabricated by deep etching processes on appropriate substrates such as glass, quartz or silicon[1]. Alternatively, hot embossing is used to structure polymer substrates. The channels are capped by anodic bonding or glue processes. Generally, these systems do not allow the reagents to be handled separately, as the channels need to be completely filled in order for the fluidics to work properly. Therefore, the application of these systems is restricted to continuous flow processes rather than batch processes as found in macroscopic laboratories.

The number of different pumping mechanisms is even greater than the number of substrate materials employed in microfluidics. Some pumping units are not an integral part of the chip and must be linked with appropriate tubes or pipes. They use e.g. piezoelectric actuation or mechanically moving parts to drive the reagents through the channels. Others take advantage of the small dimensions of the microfluidic channel itself[2]. As the chemical potentials of the channel walls and the liquid inside differ considerably, a space charge region forms at the interface. A voltage applied along the channel induces a flow at the space charge region which drags along the liquid closer to the center of the channel. This electrokinetic effect works only for narrow channels and relatively high voltages. Fluidic motion can also be induced by spatially modulating the wetting properties of a substrate. For aqueous solutions, this can be achieved by patterning the substrate with hydrophobic and hydrophilic regions. The techniques used to realize such a modulation of the wetting properties include microcontact printing[3], vapour deposition, and photolithography[4,5]. Aqueous solutions prefer to cover the hydrophilic regions and avoid to reside on the hydrophobic areas. A guided flow can be achieved[6] by changing the wetting properties with time. For example, illumination can induce a guided motion of liquids as the free energy of the surface changes locally under illumination[7]. Other pumping mechanisms include peristaltic pumps based on thin membranes[8], or polymer films[9] with a controlled deformation creating a guided flow along microchannels[10].

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Here, we would like to report on a novel approach of miniaturized liquid handling which does not move the reagents in channels but rather on the surface of piezoelectric substrates. In our case interdigital transducers excite surface acoustic waves (SAW) which transfer momentum to liquids placed on the chip. The reagents can be manipulated either as discrete droplets or by streaming patterns induced in macroscopic volumes. The technology allows both batch and continous processes to be carried out at high speed. The most important feature, however, is the programmability of the chip as different assay protocols can be realized with the same chip layout.

2. CHIP DESIGN AND FABRICATION

Figure 1a shows a 4 x 23 mm^2 $LiNbO_3$ agitation chip currently used in the AdvaCard^{TM}. The chip contains three interdigital transducers (IDTs) with 15 finger pairs each. The IDT electrodes operate bidirectional, i.e., a SAW is launched in both directions perpendicular to the fingers. One of the IDTs has an aperture of 850 µm and is shown in Fig. 1b. The electrode spacing is 12 µm generating a SAW with a wavelength of 24 µm at a resonance frequency of 155 MHz. The other two IDTs on the agitation chip have an aperture of 1.7 mm and operate at 145 MHz resulting in a SAW of 25.6 µm wavelength. The user can define various agitation patterns by alternately adressing the IDTs with the two different resonance frequencies.

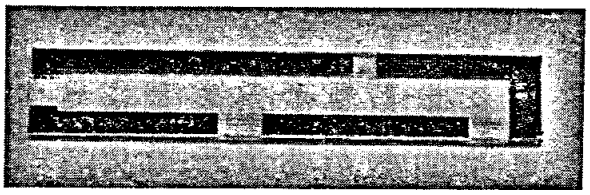

Fig 1a

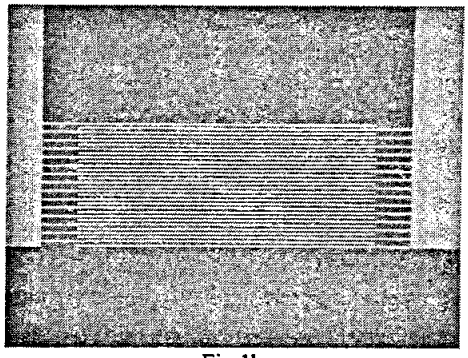

Fig 1b

Fig 1: (a) $LiNbO_3$ agitation chip with three IDT electrodes. (b) Enlargement of the narrow IDT shown in (a). The aperture is 850 µm, the SAW propagation direction is perpendicular to the fingers.

Figure 2 shows a more complex fluidics chip called "microfluidic processor". This chip is designed to move reagents along different paths defined by six IDTs: Four IDTs can drive the reagents horizontally and two vertically. The reagents can be moved across the homogenously metallized area which resides in the bottom half of the chip. This metallized area also acts as an electric contact for all IDTs which are located at its outer perimeter. In one specific design two heater elements with thermocouples are included (one of them in the upper horizontal SAW path). The IDTs are contacted by eight contact pads at the bottom of the chip. The eight contact pads at the top electrically address the heaters and the thermocouples. The chip's size is 19,5 by 20,5 mm^2.

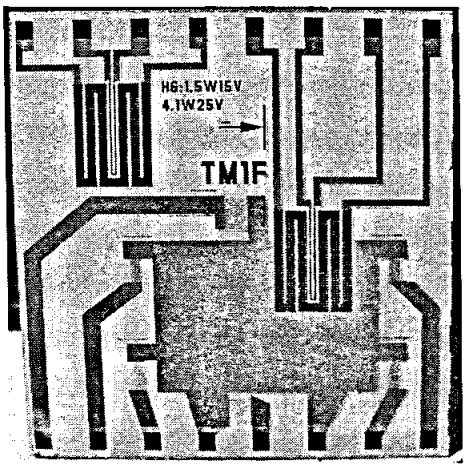

Fig. 2: The microfluidic processor for the manipulation and positioning of reagents. The reagents can be moved across the homogenously metallized area which resides in the bottom half of the chip. The contact pads for all IDTs are located on the bottom of the chip. The two meanderlike electrodes are heater elements with thermocouples that allow e.g. for an on-chip PCR.

Fabrication of the chips starts with the photolithographic definition of the electrode structures on four inch $LiNbO_3$ wafers. Here, we used an image reversal photoresist which was directly exposed to a mask aligner. A 300nm gold metallization which was followed by a lift off process defined the electrodes. Titanium was used to optimize the adhesion of the gold to the chip's surface. The metallized wafers were then coated with a 800 nm thick SiO_2 layer to provide the chips with a biocompatible and chemically stable surface. A second photolithography step defined the contact areas, at which the SiO_2 layer was removed by chemical etching in hydrofluoric acid. Finally, the microfluidic processor was homogenously silanized with octa-decyl-trichlorsilane (OTS), following a silanization procedure as described previously[11]. Employing this technique, a self assembled layer of about 2.6 nm thickness is formed on the chip's surface, resulting in a wetting angle of about 110°.

Both the agitation chip and the microfluidic processor were electrically adressed by spring contacts connected to an RF power generator. The agitation chip was integrated into a plastic card in order to agitate an area larger than the chip's size. The microfluidic processor was placed into a metallic sample holder which was temperature controlled by a Peltier element.

3. EXPERIMENTAL RESULTS

3.1. Agitation chip

In Fig. 3 we show two time series of three pictures each demonstrating the SAW-induced agitation in a capillary gap of 100µm in thickness. The lateral dimensions of the gap are 75mm by 21mm. Three agitation chips with a spacing of 22mm were integrated into the AdvaCard™ to ensure an optimal homogenization of the dye. We used bromphenol blue dissolved in water to visualize the degree of homogenization. The two rows (a) and (b) show the spreading of the dye recorded at the start of the experiment, after 2 minutes, and after 8 minutes. To start the experiment bromphenol blue was injected into four holes of 1mm diameter drilled into the AdvaCard. In the top row (a) the SAW power was on while in the bottom row (b) the SAW power was off, i.e. no active agitation mechanism was used. The agitation protocol was to switch 500mW of RF input power every 30 seconds between the 155 MHz transducer and the two 145 MHz transducers. The experiment was carried at room temperature and the microscope slide was placed directly on top of an aluminum plate for optimal heat dissipation. The temperature gradient induced by the RF power was found to be on the order of 0.1K.

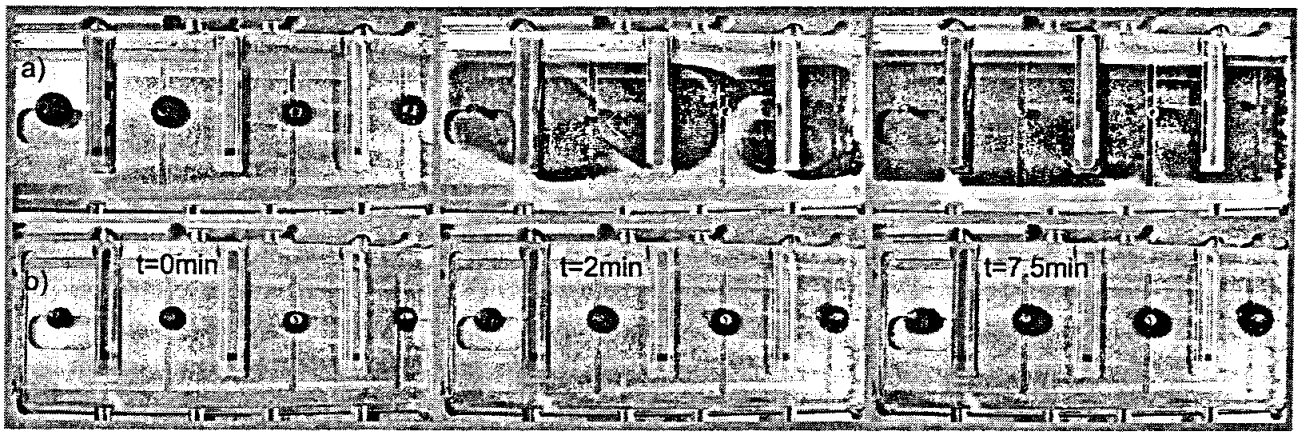

Fig. 3: Spreading of bromphenol blue with (top row) and without (bottom row) SAW agitation in a capillary gap of 100μm in thickness. The lateral dimensions are 75mm by 21mm.

SAW agitation leads to a complete homogenization of the dye after 7.5 minutes and after 2 minutes already more than 80% of the capillary gap are covered by bromphenol blue. At this point in time the SAW induced streaming patterns can be seen in the figure. The homogeneous dye distribution can only be achieved in this time frame if the streaming patterns are alternated. Otherwise there are regions in the capillary gap which are not covered by the dye. In contrast to the results achieved with SAW agitation the dye has only spread minimally in the bottom row (b). This spreading is in part due to diffusion and in part due to the flow caused by the injection of the dye. This experiment demonstrates that SAW is an efficient agitation mechanism for small liquid volumes of about 100μl. Furthermore, SAW pumps are basically two-dimensional and therefore do not add any dead volume to the system. The SAW agitation is even more efficient if the liquid is not confined in a capillary gap but in a droplet. Here, the complete homogenization of a 5μl volume was observed in seconds[12] rather than minutes. We attribute the gain in agitation efficiency to the fact that the droplet has only one boundary, defined by the agitation chip, at which the streaming velocity has to be zero. In a capillary gap, on the other hand, there are two of these boundaries leading to enhanced frictional forces. Agitation experiments in capillary gaps of 30μm, 60μm and 200μm thickness corroborate this finding as the agitation efficiency was found to increase with the thickness of the gap.

The agition chip has been designed to be integrated in a plastic mixer card (AdvaCard™). The target application for the AdvaCard™ is the agitation of sample solution during the hybridization of DNA microarrays. Generally, the substrate for DNA microarrays are conventional microscope slides with dimensions of about 75mm by 25mm. Different oligonucleotide probes with diameters of 50 to 200μm cover the microscope slides in a checkerboard pattern. The pitch is typically 300-500μm. The sample solution contains fluorescence labeled DNA strands that can bind specifically to these probes and thus be identified. During the incubation the sample solution is sandwiched between the DNA microarray and a cover slip forming a capillary gap of about 30-100μm in thickness. For low abundancy genes the immediate vicinity of the corresponding oligonucleotide spot will be quickly depleted. Without active agitation diffusion is the only mechanism for the DNA strands to be transported to their complimentary oligonucleotide spots. However, on the scale of several centimeters, diffusion is a notoriously slow process for molecules the size of the DNA strands as discussed here. It has been estimated that it would require weeks for the hybridization reaction to reach equilibrium. Given the dimensions of the capillary gap pumping excess hybridization solution back and forth is a very inefficient agitation mechanism as the Reynolds number is such that only laminar flow is possible, i.e. turbulences do not occur. Also, the pumping mechanism adds dead volume to the system and therefore reduces the sample concentration. Therefore it is preferable to induce variable laminar streaming patterns without adding any dead volume. The SAW based agitation chips fulfill this requirement as the SAW pump has a thickness of only 200nm and several pumps can be integrated on one chip allowing for different streaming patterns.

In Fig. 4 we show the results of an E. coli hybridization with the Advacard™ used for agitating the sample solution. Three sets of experiments were carried out: one without agitation, the second with laminar agitation and a third with quasichaotic agitation. For laminar agitation only one set of SAW pumps was used to induce the streaming whereas for the quasichaotic agitation both sets were alternated. As can be seen from the figure laminar agitation increases the

signal by a factor of two and quasichaotic agitation roughly by a factor of four. The enhanced signal is due to the fact that the diffusion is too slow to overcome the sample depletion in the vicinity of the spots while agitation sweeps the complimentary probes across the spots at a much faster rate.

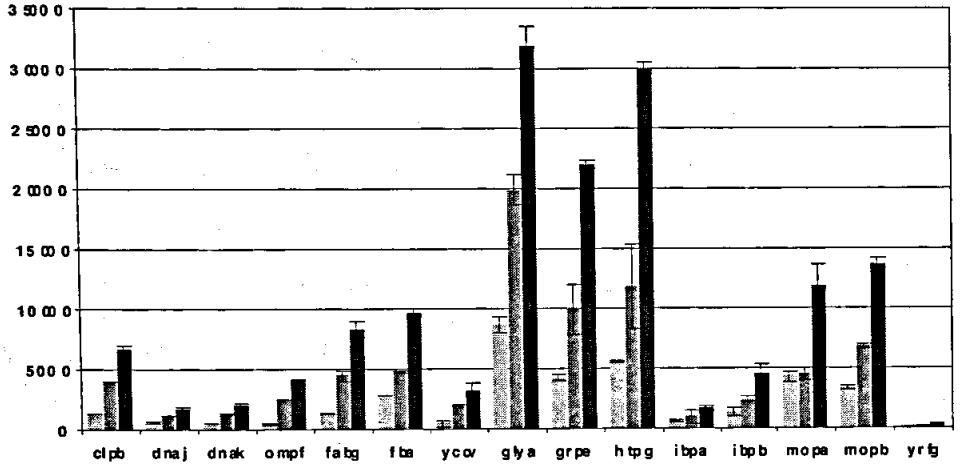

Fig. 4: Fluorescence intensity E. coli hybridized against oligonucleotides spotted on a chip. The hybridization time was set to be 1h and the incubation temperature 42°. The light bars on the left of the triplets are the signals achieved without agitation, the darker bars in the middle with a laminar agitation protocol and the black bars with a quasichaotic agitation protocol. The error bars for different replicas are also indicated.

3.2. Microfluidic processor

The microfluidic processor was designed to position and move reagents on the chip's surface. In Fig. 5 we show a time series of four consecutive pictures demonstrating that two droplets of aqueous solution can be moved towards each other and be merged subsequently. The droplets were placed between two IDTs facing each other to make the motion in opposite directions possible. The IDTs were then fed with an RF input power of about 100mW at which point they started moving. For this chip we found 100mW to be the minimum power required to overcome the pinning forces between the droplet and the chip's surface. The threshold was found to depend critically on the wetting angle between the of the droplet. For hydrophilic surfaces the value of the threshold increases dramatically. Below the threshold only internal streaming patterns can be induced but the center of mass does not move. It is important to note that the droplets move in a straight line although the chip's surface was not structured to provide additional confinement. Also, the droplets can be moved towards each other despite the fact that they travel on a path defined by two counterpropagating SAWs. After the droplets had merged the RF power of one IDT was switched off and the total volume was moved subsequently. The direction of the droplets' propagation was in all cases identical with that of the SAW. The droplets were dispensed on the chip by a Hamilton syringe. The volume of the droplets was about 300nl and 500nl, respectively.

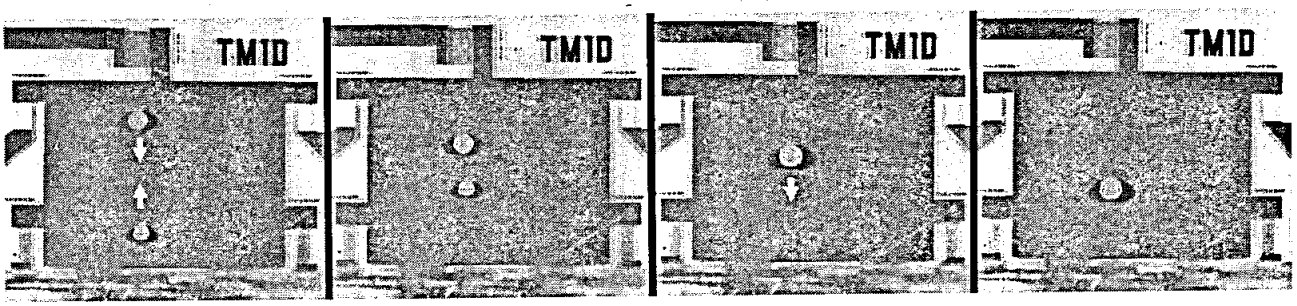

Fig. 5: Time series of two droplets (300nl and 500nl volume) being moved towards each other and merged. The direction of propagation is indicated by arrows. The last picture shows that the merged droplet can also be manipulated. Arrows indicate the direction of propagation.

In Fig. 6 we show a time series of seven consecutive pictures in which three droplets were manipulated in a similar manner as described above. The volume of all droplets was about 300nl. In the first four pictures we demonstrate that only one droplet can be moved while the others stand still. This was achieved by feeding only one IDT with the threshold input power. As the droplets move they are constantly being stirred which explains why the color reaction seen in picture 4 occurs instantaneously. In pictures 4 to 8 we demonstrate that a droplet can be moved in two perpendicular directions and then be merged with another droplet to give yet another color reaction. This chip is also not structured to provide for any confinement.

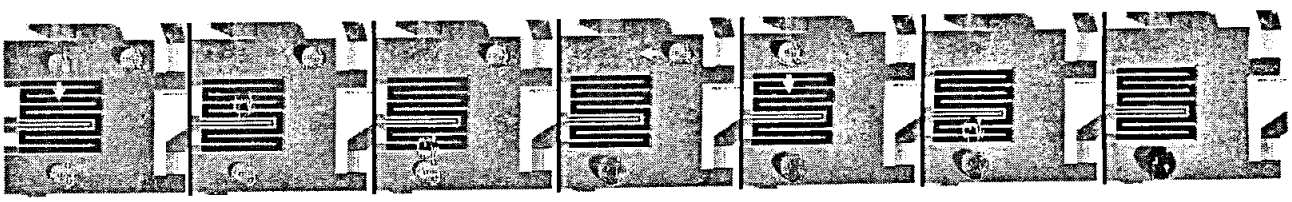

Fig. 6: Time series of three droplets (300nl volume) being manipulated. The direction of propagation is indicated by arrows Pictures 1 to 4 show how one droplet is moved and merged with another droplet to give a color reaction. Pictures 4 to 7 show how a third droplet is merged with the other to give yet another color reaction. Arrows indicate the direction of propagation.

We also determined the volume range for which droplets can be moved using SAW. We found the SAW actuation to work from a few picoliters to several microliters. However, for droplet diameters on the order of the SAW wavelength the agitation mechanism is not as efficient as for droplet diameters considerably larger than the SAW wavelength. We ascribe this finding to the fact that the SAW energy cannot be fully transferred for these droplets. The maximum velocity of the droplets was measured to be 5 cm/s. This value could be achieved by increasing the RF input power. Higher velocities are also possible but could not be measured given the limited time resolution of our video camera. By further increasing the RF power the droplets can be moved off the chip. In the experiments described above we have shown that many different reagents can be moved independently by employing different SAWs with different propagation directions and/or amplitudes. Using two counterpropagating SAWs it is possible to move two droplets towards each other.

4. DISCUSSION

The pumping mechanism for both the agitation and the motion of droplets is a momentum transfer from surface acoustic waves to the liquid on the chip's surface. SAWs can be excited by several means, most commonly using metallic interdigitated electrodes on top of piezoelectric substrates. The application of a radio frequency signal to these interdigital transducers leads to the excitation of a SAW with a wavelength defined by the finger spacing of the IDT. Typical displacement amplitudes of a SAW are in the nanometer regime. The amplitudes can be externally controlled by adjusting the excitation signal. The small surface displacement caused by the SAW can couple efficiently to liquids on top of the surface and induce acoustic streaming. Surface acoustic waves actuate liquids in a "remote control" mode

as the liquid needs not be in direct physical contact with the IDTs acting as the pumps. The SAW rather propagates across the surface and interacts with the liquids at the desired locations. The propagation of a SAW is similar to the propagation of a laser beam as surface acoustic waves diverge only minimally perpendicular to the direction of propagation. The width of the SAW beam is therefore determined by the aperture of the IDTs. As a result, the spatial resolution for placing reagents on a chip's surface is determined by the aperture of the IDTs driving the liquids. The lack of divergence also explains why some reagents can be moved while others stand still as shown in Fig. 6.

Surface acoustic waves can induce streaming patterns inside a liquid and they can move the whole liquid volume across the chip. The liquid's center of mass changes its position only if the amplitude of the SAW exceeds a critical value. This critical SAW amplitude depends on the frictional forces, the volume of the droplet and the chip's surface properties which can be understood in terms of the pinning at the three-phase boundary between the liquid, the solid surface and the surrounding gas. Other properties of the liquid such as the viscosity were found to have only a small impact on the threshold SAW amplitude. We found the threshold value to be very reproducible for a given set of parameters. Furthermore, surface acoustic waves can be efficiently blocked in their direction of propagation as the waves are strongly attenuated by the interaction with liquids. As a result two reagents can be moved in opposite directions on the same acoustic path as demonstrated in Fig. 4. Here, one droplet provides an "acoustic shadow" for the other. As surface acoustic waves are confined to a layer of about one acoustic wavelength measured from the IDT's surface they are only minimally attenuated by coatings which are thin compared to the wavelength. Therefore the SAW pumping mechanism also works for coated IDTs which can be desirable to ensure biocompatibility or to provide a defined linker chemistry. As typical wavelengths for SAW actuation are between 1 and 100μm one has considerable flexibility with respect to the thickness of surface coatings.

It was found that for certain ratios between the IDT aperture and the droplet's diameter the liquid left the SAW path and came to a standstill. This effect could be overcome by structuring the chip's surface such that the droplet was guided by hydrophilic tracks as described previously[12]. In this case, the width of the tracks must be chosen such that the pinning forces can still be overcome. Typical values for the width of the tracks were found to be in the range of 10% to 30% of the droplet's diameter. The fluidic tracks keep the liquids confined to the SAW propagation path as long as the SAW amplitude does not exceed a second critical value. Lateral structuring of the chip's surface with hydrophilic and hydrophobic regions can also be used to position the droplets more reproducibly. If e.g. an aqueous reagent is to be moved to a defined spot on the surface a hydrophilic anchor can be used to pin the liquid to this spot. The reagent can then only be removed if the SAW amplitude exceeds the second threshold value.

5. OUTLOOK

It should be noted at this point, that the very same SAW technology described here can also be used for sensor applications[13]. SAW sensors are generally based on the sensitivity of SAWs to loading a free surface with a finite mass. For such a sensor design two IDTs of identical resonance frequency are placed on opposite ends of the chip and the phase shift and/or the attenuation is measured as a function of the mass loading. Based on this principle an integrated feedback system can be designed with one SAW moving a droplet from one location to another, while the transmission of a second SAW is used to determine whether the droplet has arrived at its designated destination. The same IDTs can be used for both actuation and detection. The only difference is that for actuation the required RF input power exceeds the one for detection by more than an order of magnitude. Even more sophisticated sensors can be designed[14] resulting in special IDT structures called slanted transducers. These IDTs transmit a relatively narrow SAW beam with the specific point of excitation defined by the applied SAW frequency. This narrow SAW beam can be swept along the whole aperture of the slanted transducer by modulating the excitation frequency. Also, a two-dimensional spatial resolution is possible using two sets of slanted transducers with two perpendicularly propagating SAWs. The principle of such two-dimensional SAW sensors has been shown to be sensitive to the presence of free charges close to the surface and mass loading effects[15]. Of course, such slanted transducers can also be employed for pumping and agitating liquids. Here, the spatial tunability of the sound path gives additional flexibility for multiplexing different fluidic circuits.

The feedback system described above would also make it possible to encapsulate the whole microfluidic processor without losing process control. For biochips this encapsulation may be necessary to reduce contamination and to control

reaction parameters such as the relative humidity and the temperature. For specific applications, it is possible to cool or heat parts of the microfluidic processor, e.g. by using meander-like on-chip heaters or Peltier elements. To keep the reagents from evaporating they can be covered by an oil film which can be placed on the desired reagents by using SAW pumps.

6. SUMMARY

In summary, we have realized two different chip layouts to demonstrate the basic features of SAW induced liquid handling systems. The agitation chip was shown to stir even smallest amounts of liquids effectively. This programmable agitation can be used to induce and accelerate physical, chemical or biological reactions. Furthermore, we have investigated the properties of a programmable microfluidic processor. Here, we used the surface tension to confine the liquids under investigation to specific volumes. The independent and parallel movement of different droplets was achieved by employing different acoustic waves. It was found that the SAW technology allows reagents to be placed on any desired spot on the chip's surface. Furthermore, reagents can be manipulated independently and in parallel. The SAW liquid handling system is scalable over several orders of magnitude as the pumping mechanism has been found to work for volumes as small as several picoliters up to several microliters. Our experimental results suggest that the acoustically driven liquid handling on a free surface has the potential to define a new generation of programmable microfluidic systems.

REFERENCES

1. J. G. Smits, *Sensors and Actuators* **A21-23**, 203 (1990).
2. A. Manz, C. S. Effenhauser, N. Burggraf, D. J. Harrison, K. Seiler and K. Fluri, *J. Micromech. Microeng.* **4**, 257 (1994).
3. G. P. Lopez, H. A. Biebuck, C. D. Frisbie, G. M. Whitesides, *Science* **260**, 647 (1993).
4. G. Möller, M. Harke, H. Motschmann, *Langmuir* **14**, 4955 (1998).
5. H. Gau, S. Herminghaus, P. Lenz, R. Lipowski, *Science* **283**, 46 (1999).
6. S. Daniel, M. K. Chaudhury, J. C. Chen, *Science* **291**, 633 (2001).
7. K. Ichimura, S. K. Oh, M. Nakagawa, *Science* **288**, 1624 (2000).
8. R. M. Moroney, R. M. White, R. T. Howe, *Appl. Phys. Lett.* **59**, 774 (1991)
9. M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer, S. R. Quake, *Science* **288**, 113 (2000).
10. W. L. Nyborg: *Acoustic Streaming*, in *Physical Acoustics* 2B, Ed. W.P. Mason, Academic Press 265, (1965).
11. D. E. Kataoka, and S.M. Troian, Nature **402**, 794 (1999).
12. C. Strobl, A. Rathgeber, A. Wixforth, C. Gauer, J. Scriba, to be published in *2002 IEEE Ultrasonics Symposium Proceedings*
13. *Acoustic Wave Sensors: Theory, design, and physico-chemical applications,* D. S. Ballantines et al., eds. (Academic Press, San Diego, London, 1997).
14. M. Streibl, F. Beil, A. Wixforth, C. Kadow, A. C. Gossard, *IEEE Ultrasonics Symposium Proceedings*, 11 (1999).
15. M. Streibl, A. Wixforth, J.P. Kotthaus, A.O.Govorov, C. Kadow, A.C. Gossard, *Appl. Phys. Lett.* **75**, 4139 (1999).