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ORIGINAL PAPER

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Ultrasonic agitation in microchannels

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Abstract This paper describes an acoustic method for inducing rotating vortex flows in microchannels. An ultrasonic crystal is used to create an acoustic standing wave field in the channel and thus induce a Rayleigh flow transverse to the laminar flow in the channel. Mixing in microchannels is strictly diffusion-limited because of the laminar flow, a transverse flow will greatly enhance mixing of the reactants. This is especially evident in chemical microsystems in which the chemical reaction is performed on a solid phase and only one reactant is actually diffusing. The method has been evaluated on two different systems, a mixing channel with two parallel flows and a porous silicon micro enzyme reactor for protein digestion. In both systems a significant increase of the mixing ratio is detected in a narrow band of frequency for the actuating ultrasound.

Keywords Microchannels · Rotating vortex flow · Rayleigh flow · Acoustics · Ultrasonic agitation · Reagent mixing

Introduction

Two parallel flows, injected separately in a microfluidic channel, will stay separated and mix very slowly, because of the extreme laminar flow conditions that commonly exist in microdomain. This has been described several times and is even utilised in some applications in which the diffusion conditions at the interface have been used to control a reaction [1, 2, 3]. In most microfluidic systems wherein chemical reactions take place, however, one of the major issues is the time required for the reactants to make contact with each other and to mix well. The problem arises because of the often very low Reynolds number in microsystems, due to the small dimensions. This leads to a laminar flow, which makes any transport transverse to the main flow strictly diffusion-limited. Various alternatives for mixing the components have been presented; these use different channel designs [4, 5, 6, 7, 8], movable parts [9], or ultrasonic mixing chambers [10, 11]. A drawback with these solutions is that the mixing often is separated from the rest of the system. Therefore, the system might require redesigning and this can increase the dead volume of the system. This is especially evident in chemical microsystems in which the chemical reaction is performed on a solid phase. A reason for performing the chemistry with one of the reactants, e.g. an enzyme, immobilised on a stationary phase and the substrate is supplied via the mobile phase is that high substrate turn-over rates can be obtained and thus rapid analytical procedures can be developed [12, 13]. When performing enzymatic reactions in flow-through format, e.g. in a flow-injection analysis (FIA) system, using open tubular microreactors with a catalytic species immobilised on the channel wall, the rate-limiting factor will be the diffusion constants of the substrate and the products to and from the channel wall. In the microdomain, as reasoned above, the slowest sample transport is in the vicinity of the channel wall, where the chemical reaction takes place. This is a limitation when it comes to reducing the retention time, a desirable task in the screening processes commonly used today.

One possible way of speeding up the process could be to induce a vortex flow in the channel to carry reactants from the centre flow to the channel walls, and the products back in the opposite direction.

Lateral acoustic flow, or Rayleigh flow [14], is a means of inducing such vortices. Rayleigh flow typically occurs in the presence of a acoustic standing wave field in a channel with a width much smaller than the wavelength of the acoustic wave but much larger than the thickness of the periodic boundary layer. The streaming velocity, u_s, at the channel wall, just outside the boundary layer, is given by Raleigh's law [15]:

$$u_s = \frac{-3}{4\omega} V(x) \frac{\mathrm{d}V(x)}{\mathrm{d}x}$$

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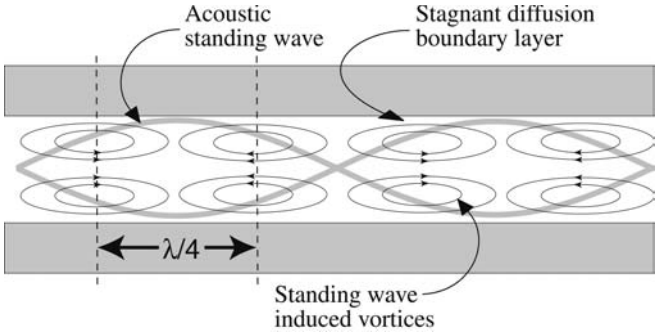

Fig. 1 Schematic diagram of how an acoustic standing wave field, in a sufficiently narrow channel, induces several vortices spaced $\lambda/4$ apart

where $V(x)$ is the acoustic particle velocity outside the boundary layer and ω is the frequency of the sound field. This slip velocity, u_s, is directed toward the velocity nodes of the standing wave. In the centre of the channel this flow is counteracted by a flow in the opposite direction, resulting in rotating vortex flows transverse to the acoustic field on both sides of the channel, spaced $\lambda/4$ apart [15], Fig. 1.

This phenomenon has rarely been used in microtechnology, with few exceptions. Vortex flow has been used to enhance heat transfer between two parallel plates [16]. The possibility of using the phenomenon to enhance mass transfer in a bioseparator for protein extraction is discussed in theory elsewhere [17].

By use of high-frequency ultrasound it is possible to achieve an acoustic wavelength of the same dimension as most microfluidic channels; it should, therefore, be possible to induce the discussed Rayleigh flow in any well-defined microchannel system.

The ability to induce acoustic flow to enhance chemical reaction rate has been utilised, although on a macro scale, in several different applications such as sonochemistry [18, 19, 20] which uses ultrasonic horns to form a concentrated sound field and induce acoustic streaming and cavitation bubbles with high-effect ultrasound.

The possibility of using ultrasound to pump liquids with surface acoustic waves has also been shown [21, 22, 23]; this technique has recently been commercially available (ArrayBooster, Advalytix)

To study the feasibility of using Rayleigh flow for agitation and breaking up laminar flow lines in microchannels this paper describes a test channel designed for performing mixing experiments in microfluidic channels. As an application suitable for this kind of micro mixing a system previously reported by our group, a parallel channel enzyme microreactor in porous silicon [24, 25], was chosen and the Rayleigh flow-induced agitation was evaluated.

Materials and methods

All structures were fabricated in (110)-silicon (p-type 20–70 Ohm cm) with anisotropic wet etching in KOH (40 g/100 mL H_2O, 80 °C).

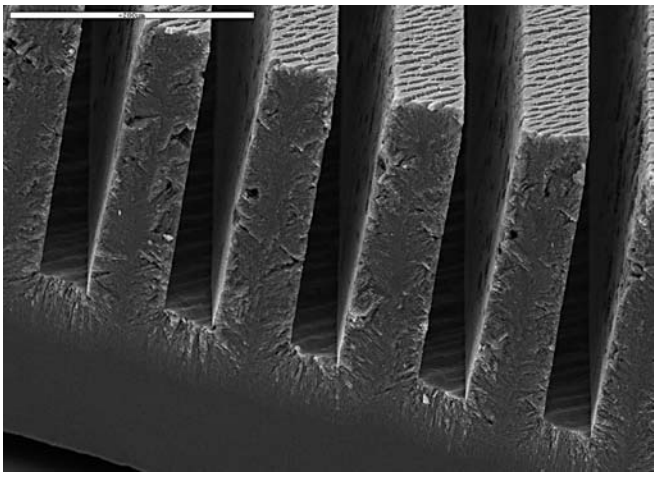

Fig. 2 Cross view of a high-aspect-ratio parallel-channel enzyme reactor in porous silicon. Each channel is 25 µm wide and 300 µm deep with a 75 µm separating wall in between

The test channel was 30 mm long, 300 µm deep and 75 µm wide with parallel vertical walls. The two flow paths stream in parallel with a 5- µm dividing wall, in order to develop strict laminar flows in each of the inlet fluids, before uniting into one common channel.

The porous silicon enzyme reactor comprised 32 parallel channels, 10 mm long, 300 µm deep and 25 µm wide, Fig. 2. The silicon was anodised in an HF–dimethylformamide (DMF) solution (mixing ratio 1:1), with a current density of 50 mA cm^{-2}, to obtain a porous surface layer. The DMF content of the electrolyte enables masking of the porous layer with SiO_2 [26], preserving the polished silicon surface for subsequent anodic bonding of a glass lid to the micro reactor structure.

The channels were designed to form an acoustic resonator in the vertical direction of the chip, i.e. a standing wave was created between the channel bottom and the Pyrex lid. Because the test channel and the microreactors were fabricated on different occasions using time etch stop as the end criterion for the etch process the height of the channels in the different structures varied slightly around 300 µm.

The structures were sealed with an anodically bonded Pyrex lid and the flow was injected via capillaries glued into the chip. An ultrasonic (US) crystal was coupled to the silicon chip (an aqueous gel was used to ensure a good acoustic contact) and a polyurethane wafer was used as backing to the US element, Fig. 3. The polyurethane backing served as an acoustic terminator to minimize power dissipation from the microsystem.

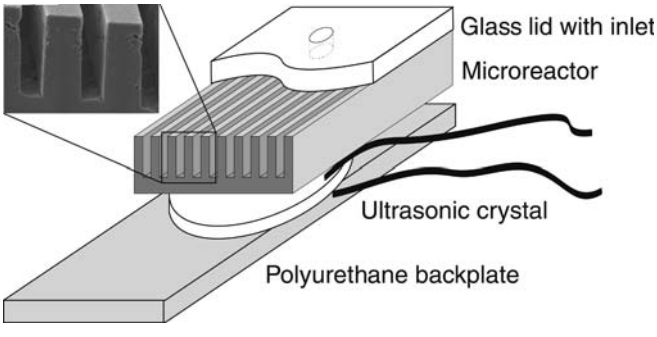

Fig. 3 Schematic view of the set-up with the enzyme reactor, ultrasonic crystal, and polyurethane back plate

The height of the channels in both the test structure and the microreactors, 300 µm, was selected to correspond to the acoustic wavelength of the standing wave of approximately 5 MHz. Standard ultrasonic crystals with a resonance frequency of 6 MHz were used. The reason for using crystals with higher resonance than the operating frequency used was to avoid any influence from the frequency characteristics of the crystal, i.e. for lower frequencies the output power of the US element is less frequency-dependant, being operated in the flat band area of its impedance spectra. The voltage applied to the crystal was kept constant throughout the measurement at 10 V_{pp} (peak-to-peak).

The mixing efficiency was initially tested with a colorimetric assay. In the test channel liquids at two different pH, 2.5 and 6 respectively, were injected, one containing a pH reagent, dinitrophenol (Merck, Darmstadt, Germany). The actuating frequency was scanned and the absorbance shift of the fluid, resulting from the change in pH, at the output was monitored with an absorbance meter (Waters 486, Milford, MA, USA). To avoid gas-bubble formation all liquids were degassed ultrasonically before injection.

In the enzyme reactor trypsin (Sigma, St Louis, MO, USA) was immobilised on the porous silicon matrix in a three-step procedure – silanisation, glutaraldehyde activation, and enzyme coupling. A detailed description of the immobilisation procedure is given in Ref. [27]. To evaluate the enzymatic turnover benzoyl-arginine-ethyl-ester (BAEE; Sigma) was pumped through and past the reactor at different concentrations and flows rates. The resulting absorbance shift, because of the digestion of BAEE, was monitored with the same set-up as above. By turning the ultrasound on and off the agitation effect of the acoustic streaming was measured. Because enzymatic reactions are strictly dependent on the temperature, a Pt-100 element was placed in the flow monitoring the temperature of the reactor output.

Results and discussion

Rayleigh flow-induced mixing in the test channel

A drawing of the test channel for the initial investigation of mixing efficiency using Rayleigh flow induced by ultrasonic standing waves is seen in Fig. 4. The flow divider was introduced to ensure vertical flow lamination of the two fluids (A and B in the drawing) in the high-aspect-ratio channel.

First a calibration plot was made with no ultrasound actuation. The flow was varied from 1 to 160 µL min^{-1} and diffusion mixing was measured as the difference between the absorbance of the unmixed pH indicator and the mixed species. To determine the absorbance at complete mixing, 1 mL of each liquid was left in a vial for 30 min and the result injected in the absorbance meter.

Second, to establish the resonant frequency of the test channel and the micro enzyme reactor the flow was maintained constant and the frequency of the ultrasonic actuator was varied while monitoring the absorbance shift due to mixing.

Finally, to evaluate the mixing efficiency of the ultrasound the actuator was switched on and off at different flow rates. The frequency of the actuator was fixed at the resonance frequency of the corresponding microstructure.

When scanning the actuating frequency using the test channel, the absorbance showed a significant absorbance peak for a frequency of 4.85 MHz, Fig. 5. The bandwidth of the peak seemed to be very narrow and no effect was observed for neighbouring frequencies. The peak frequency corresponded very well to the measured height of the channel, 305 µm.

The absorbance, monitored at an excitation frequency of 4.85 MHz, for different flows is shown in Fig. 6. The mixing was found to be effective at flow rates lower than approximately 20 µL min^{-1}, corresponding to an average linear flow velocity of 14.8 mm s^{-1}. At flow rates higher than 40 µL min^{-1} (29.6 mm s^{-1}) the retention time in the channel was so short (≤1 s) that the two fluids were not exposed to Rayleigh flow mixing for sufficient time to en-

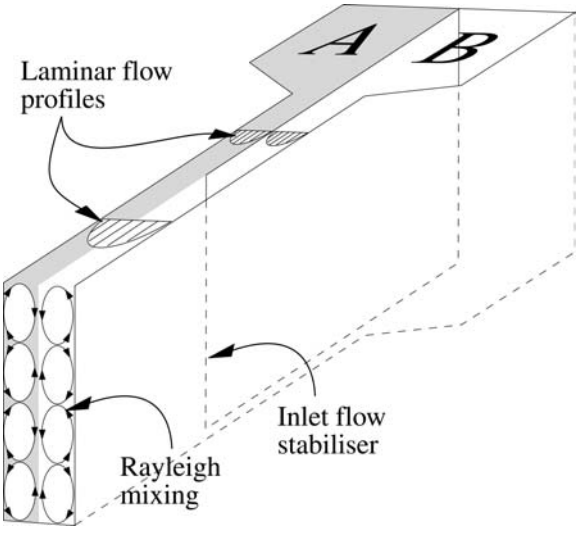

Fig. 4 Drawing of the test channel. The flow divider was introduced to ensure vertical flow lamination of the two fluids (A and B in the drawing) in the high-aspect-ratio channel. The acoustic standing wave was formed between the channel bottom and the Pyrex lid transverse to the flow

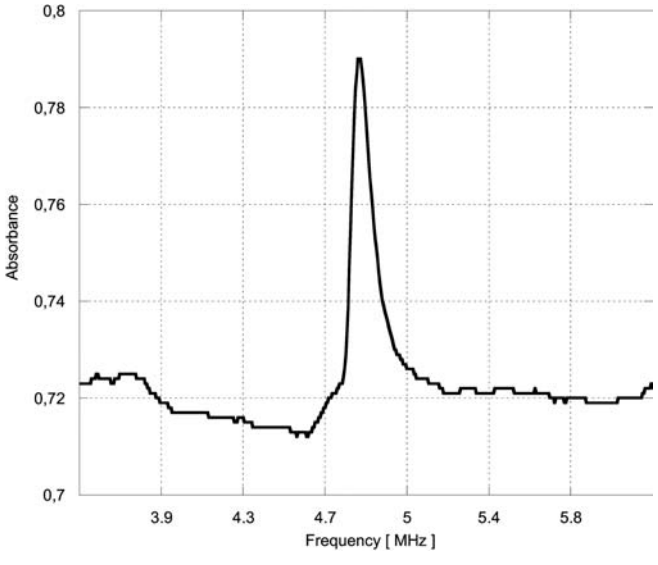

Fig. 5 Absorbance at 261 nm, measured at the outlet of the test channel, for different frequencies of ultrasonic agitation and a flow of 10 µL min^{-1}

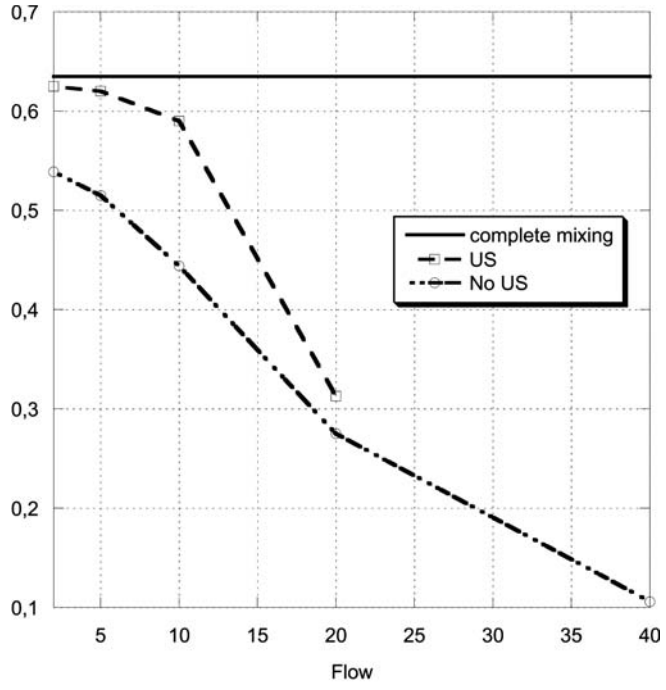

Fig. 6 Absorbance at 261 nm, as a indication of the efficiency of mixing, measured at the outlet of the test channel for different flow rates, with and without ultrasonic agitation

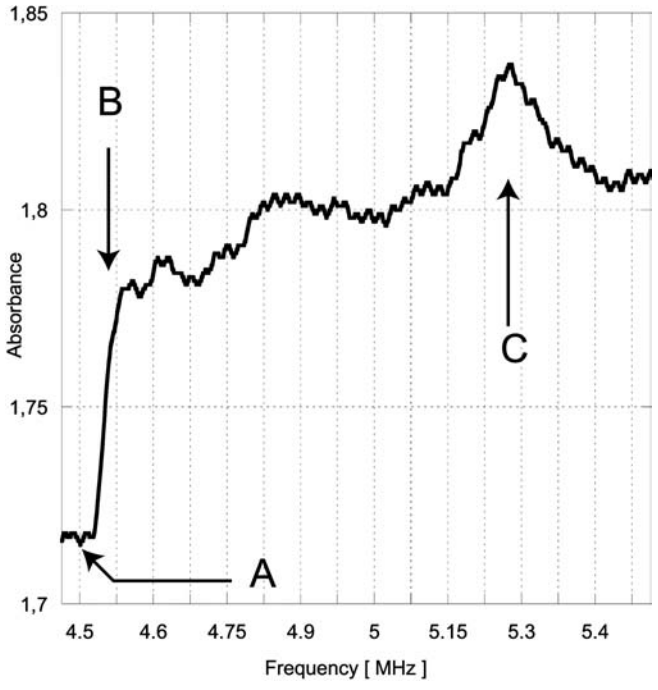

Fig. 7 Absorbance at 251 nm, measured at the outlet of an enzyme reactor digesting 0.5 mmol L^{-1} BAEE at a flow rate of 16 µL min^{-1}: (*A*) ultrasound power switched off, (*B*) ultrasound power switched on, (*C*) Rayleigh flow-induced mixing

able efficient mixing; for flow rates lower than 4 µL min^{-1} (2.96 mm s^{-1}, approximate retention time ≥10 s) almost full mixing was accomplished.

Rayleigh flow-induced agitation in the enzyme reactor

It should be noticed that a significant increase in the catalytic output of the microreactor was observed as soon as the acoustic power was switched on even though the system was not fully tuned to the resonance frequency (see insert arrow B in Fig. 7). The increase corresponded well to the frequency characteristics of the ultrasonic crystal, i.e.:

- it was constant at frequencies below 6 Mhz (except for frequencies matching the criteria for Rayleigh flow in the microchannels);
- it displayed a small peak at the resonance frequency of the crystal at 6.1 MHz;
- it finally decayed at higher frequencies.

As the acoustic frequency was scanned from lower frequencies a clear increase in catalytic turnover was observed at 5.25 MHz, which matched the resonance mode for the vertical direction of the microreactor channels (insert arrow C, Fig. 7). The periodic oscillation that is seen in Fig. 7 was caused by minute periodic variations in flow rate induced by the syringe pump. This also illustrated the sensitivity of the system with regard to the rate of transport of substrate to the microreactor surface.

A small peak was also registered at approximately 7.6 MHz (not shown), corresponding to the frequency where 1.5λ equalled the channel height, forming a Rayleigh flow with four additional vortices in the channel. Because this frequency was higher than the ultrasonic crystal resonance the acoustic output power was very low and the catalytic turnover peak was hardly distinguishable over the noise.

The measured increase in catalytic effect with acoustic agitation at resonance was estimated to be between 15 and 20%, Fig. 8. It can also be noted that the resonant peak bandwidth, Fig. 7, seems larger than for the test channel set-up used in Fig. 4. This might be caused by the porous matrix, because it is possible for the acoustic wave to reflect at several depths in the porous silicon matrix. This indicates that the porous surface is a non-perfect reflector of the acoustic wave, which in turn might cause a damping effect. This, combined with the larger volume of the reactor compared with the test channel, indicates that with higher ultrasonic power it might be possible to achieve much better agitation.

It is notable that for the enzyme reactor enhancement in digestion with ultrasonic agitation was clearly visible even though the actual linear flow rate in each channel was much lower than in the test channel, Fig. 8. This is because the microreactor system is based on immobilised chemistry in which the substrate, BAEE, has to diffuse to the solid surface of the reactor wall where the catalysis takes place. The laminar flow provides very slow transport of new substrate to the catalytic surface, which quickly results in substrate depletion in the vicinity of the immobilised enzyme. Consequently, any mixing mechanism that increases the substrate supply rate to the enzyme will have

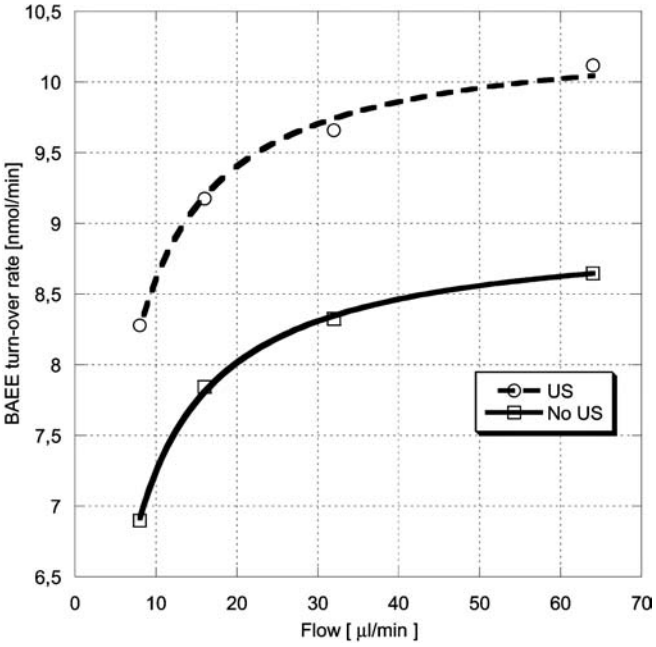

Fig. 8 Enzymatic turnover in a reactor digesting 1 mmol L^{-1} BAEE, at different flow rates, with and without ultrasonic agitation

a clear effect on microreactor turnover. Rayleigh flow-induced vortices are, therefore, an excellent means of agitating a microreactor based on an open tubular format. The micro vortices bring substrate from the rapid flow regime in the parabolic flow profile to the catalytic surfaces. The effect of acoustic mixing is therefore more pronounced in the microreactor than in the mixing experiments with the test structure. Furthermore, the active molecule, BAEE, is much larger than the pH indicators used in the test channel and, consequently, diffuses much more slowly, which in turn makes the microreactor system more responsive to mixing induced by transverse flow, i.e. micro vortices. The micro agitation effect is probably even more noticeable for heavier molecular weight substrates such as large proteins, because the diffusion constant for such molecules are significantly lower and thus the gain in substrate turnover with a guiding lateral flow will be larger.

One issue of interest is the increase in catalytic effect when the acoustic power is turned on at frequencies outside the Rayleigh flow resonance (arrow insert B Fig. 7). Because this effect could not be registered in the measurements on the test channel, the effect was first believed to be a heating effect, because of absorption of acoustic energy by the silicon. Temperature measurements at the reactor outlet, however, showed no correspondence between output temperature and ultrasonic effect. It was thus concluded that the increased acoustic energy in the microfluidic system gave rise to the increased catalytic output outside resonance.

One of the main advantages of the proposed agitation method is the ability to introduce mixing without increasing the dead volume of the system. The limitation is the high-aspect-ratio channel required, a channel design not suitable for all applications. It is also necessary that the channel material provides good acoustic contact and has low acoustic absorbance.

The structures in the study actually have some roughness at the channel bottom, because of the anisotropic etching orientation of the stop-etch planes, <111>-planes, in the silicon, which probably reduces the ideal properties of the acoustic standing wave field. This situation can be considerably improved by using other etching techniques such as DRIE, which would smoothen the channel bottom. Then the quality of the standing wave might increase further while the bandwidth of the resonant frequency peak would most probably become narrower.

One way to increase the mixing effect further with this method would be to choose a higher ultrasonic frequency, such that the channel height corresponds to 1.5λ of the standing wave, or higher orders, thus inducing as many as four vortex flows. However, to accomplish this, ultrasonic elements with a higher resonance frequency are needed.

Conclusion

A method of introducing lateral vortex flow, Rayleigh flow, in microchannels is demonstrated. The ability to induce such vortex flow acoustically makes it possible to increase mixing and convective transport in microchannels without redesigning an existing system. The acoustic mixing method is especially suitable for microsystems based on open tubular-type immobilised enzyme microreactors, because transport of substrate to the catalytic solid phase is greatly enhanced by the micro vortices generated by Rayleigh flow.

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