Artificial cilia for active micro-fluidic mixing†

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In lab-on-chip devices, on which complete (bio-)chemical analysis laboratories are miniaturized and integrated, it is essential to manipulate fluids in sub-millimetre channels and sub-microlitre chambers. A special challenge in these small micro-fluidic systems is to create good mixing flows, since it is almost impossible to generate turbulence. We propose an active micro-fluidic mixing concept inspired by nature, namely by micro-organisms that swim through a liquid by oscillating microscopic hairs, cilia, that cover their surface. We have fabricated artificial cilia consisting of electro-statically actuated polymer structures, and have integrated these in a micro-fluidic channel. Flow visualization experiments show that the cilia can generate substantial fluid velocities, up to 0.6 mm s⁻¹. In addition, very efficient mixing is obtained using specially designed geometrical cilia configurations in a micro-channel. Since the artificial cilia can be actively controlled using electrical signals, they have exciting applications in micro-fluidic devices.

Introduction

Micro-fluidics is the science and technology of manipulating and analyzing fluid flow in structures of sub-millimetre dimensions.¹–³ This field is particularly relevant for the development of lab-on-chip devices, which can be pictured as credit-card-sized fluidic systems containing tiny channels and chambers in which processes such as mixing and routing of the liquids, and separation, reaction, and detection of individual components present in these liquids are integrated. In this way, a complete large-scale analysis laboratory is miniaturized and combined on a single chip.

Many different physical phenomena can be used to manipulate fluids on sub-millimetre scale. Small droplets can be manipulated by actively changing their surface tension, for instance using electrical potentials.⁴ Fluid can be transported through micro-channels by electro-osmosis, in which a spontaneously occurring charged surface layer is set into motion by an applied electrical field.⁵ Other physical principles that can be exploited in micro-fluidics are acoustic streaming,⁶ optical manipulation,⁷ dielectrophoresis,⁸ magnetophoresis,⁹ and thermophoresis.¹⁰ Use has also been made of micro-fabricated valves to control flow in micro-fluidic channels.¹¹

Micro-fluidic mixing

A special challenge in micro-fluidic systems is to create efficient mixing flows. Due to the small channel sizes, the Reynolds number is generally low and flows are non-turbulent.¹² On the other hand, the channel size is often too large for molecular diffusion to be effective in mixing within a reasonable time, which is reflected by the Péclet number being large.¹³ To obtain efficient mixing, special strategies must therefore be followed. An approach is to create repeatedly stretching and folding flow patterns, leading to so-called chaotic advection that causes effective mixing.¹⁴–¹⁶ Nguyen & Wu¹⁴ give a review and classification of the relatively large number of micro-mixers proposed previously in the literature.

The existing micro-mixers can be divided into two general classes, namely passive and active micro-mixers. Passive micro-mixers do not require external energy, and the mixing process relies entirely on chaotic advection or diffusion. The effect is often achieved by special geometrical features like channel shape or corrugations on the channel walls. An early example is the branching channel structure proposed by Bessoth et al.,¹⁷ in which the splitting and rearrangement of fluid streams in the branching structure leads to lamination and mixing. The three-dimensional serpentine-shaped micro-channel proposed by Liu et al.¹⁸ results in chaotic advection and good mixing for relatively high Reynolds numbers. Another concept of passive micro-mixing was proposed by Song et al.,¹⁹ who used a two-phase liquid system consisting of aqueous droplets in an oily carrier liquid. By transporting the droplets through winding micro-channels, an internal flow field is generated inside the droplets and fast mixing occurs within the droplets. A well-known and elegant concept uses grooves or ridges on the channel walls, as described by Strouck et al.²⁰ For these passive groove-type mixers, the channel floor is covered with grooves of specific size and layout, inducing transverse flow patterns in the fluid as it is pumped through the channel for example by an external pump. The groove patterns are organized in segments, with neighboring segments having an alternating groove layout. A pair of consecutive segments form a “cycle”. The repeating action of alternating transverse flow patterns in subsequent
segments leads to exponentially fast mixing within the channel. One specific example is the staggered herringbone mixer, named after its herringbone-patterned grooves on the channel floor, in which complete mixing is achieved after four to ten cycles, depending on the flow conditions.20

Active micro-mixers use the disturbance generated by an external field for the mixing process, and thus they require external energy. Lu et al.21 integrated micromachined magnetic-bar mixers in micro-fluidic channels and chambers. These are rapidly rotated within the fluid by applying a rotating external magnetic field, which results in reasonably effective mixing. Another active mixing concept, proposed by Glasgow & Audry,22 is to apply sinusoidal pressure pulses to the micro-channel through the channel inlets. This causes an oscillating flow velocity which results in chaotic advection patterns. Electrical effects can also be used for micro-mixing. El Moctar et al.23 describe a micro-fluidic mixer based on the electro-hydrodynamic force present when the fluids to be mixed have different electrical properties and are subjected to an electrical field. Integrated electrodes are used to create an electric field perpendicular to the interface between the fluids in the micro-channel, resulting in a transversal secondary flow that causes mixing. Sasaki et al.24 used meandering electrodes in the micro-channel floor to induce a transversal AC electro-osmotic flow by which rapid mixing was achieved. As a final example we mention the work of Srithanan et al.,25 who described a micro-mixing concept based on acoustic streaming, in which surface acoustic waves generated by a piezoelectric transducer induce transversal mixing flow patterns.

Besides the micro-mixer concepts mentioned above, many more have been published and a more complete overview is presented in Nguyen & Wu.26 A direct comparison between the various mixers is difficult and should be made with caution. A concept may have particular advantages for specific applications, which may balance possible disadvantages. An obvious advantage of all passive mixers is that they use no external energy. A disadvantage may be that the mixing effect can not be switched on or off at will. For active micro-mixers, the energy required for their operation is a disadvantage. Also, active mixers often require complex fabrication and it is often hard to integrate them in micro-fluidic systems. On the other hand, active mixers can be switched on or off, offering more control over the process.

Although a direct comparison is difficult, the various micro-mixing concepts can be characterized using a number of characteristic parameters. Following Nguyen & Wu,24 the “operation point” of a micro-mixer is characterized by the typical values of Reynolds number $Re$ and Péclet number $Pe$ at which they have been shown to work. Other characteristics, of practical interest, are the typical mixing volumes and times, as well as the typical lateral mixing dimension (i.e., mixing length for a channel flow). A practical measure of mixing efficiency is the mixing time per volume of liquid: the smaller this value, the more effective is the micro-mixer. For the micro-mixers briefly described above, these characteristic parameters are given in Table 1. Later, we will use these data to position the new micro-mixer proposed in the present paper.

### Cilia: micro-fluidics in nature

Besides the aforementioned man-made technological solutions for micro-fluidic manipulation, we noticed that also other inhabitants of this world have evolved to manipulate fluids at small length scales. Many microscopic organisms use cilia or flagella to propel themselves through a liquid.27 Cilia can be viewed as small hairs or flexible rods (with a typical length of 10 μm and a diameter of about 250 nm) covering the outer surface of the micro-organism, see Fig. 1. An individual cilium makes an oscillating motion that is asymmetric, producing an effective stroke and a recovery stroke, with a frequency between 10 and 30 Hz.28,29 The collective beating of the thousands of cilia is very effective in generating flow: the swimming speed of Paramecium, for example, can be approximately 1 mm s$^{-1}$.30

Darnton et al.31 attached flagellated bacteria to a solid surface to create an active “bacterial carpet”. These carpets moved fluids over a depth of 50 μm, whereas the speed of the fluid just above the carpet was around 15 μm s$^{-1}$. Tracer beads revealed complex flow patterns. Dreyfus et al.32 made a flagella-like filament using magnetic particles linked by DNA, attached it to a red blood cell, and showed that this structure could propel itself through a liquid, in an oscillating magnetic field. Evans et al.33 manufactured nanorods from a composite material consisting of a rubbery matrix and superparamagnetic particles, organized in arrays on a substrate, and they could actuate these “biomimetic cilia” using a moving permanent magnet.

### Scope

Inspired by nature, we have developed electrostatically actuated artificial cilia, consisting of polymer-based micro-actuators, that

<table>
<thead>
<tr>
<th>Reference</th>
<th>$Re$</th>
<th>$Pe$</th>
<th>$V_{mix}$/μL</th>
<th>$T_{mix}$/ms</th>
<th>$L_{mix}$/mm</th>
<th>$(T/V)_{mix}$/s μL$^{-1}$</th>
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<tbody>
<tr>
<td>Passive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bessoth et al.27</td>
<td>0.07</td>
<td>60</td>
<td>0.6</td>
<td>15</td>
<td>10</td>
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<td>70000</td>
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<td>30</td>
<td>6</td>
<td>0.1</td>
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<tr>
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<td>5000</td>
<td>0.0002</td>
<td>2</td>
<td>0.5</td>
<td>10</td>
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<tr>
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<td>2000–900000</td>
<td>0.1–0.24</td>
<td>70000–200</td>
<td>7–17</td>
<td>700–1</td>
</tr>
<tr>
<td>Active</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
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<td>105</td>
<td>0.2</td>
<td>60000</td>
<td>3</td>
<td>300</td>
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<td>3000</td>
<td>0.05</td>
<td>1000</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
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<td>1050</td>
<td>0.02</td>
<td>100</td>
<td>0.25</td>
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<td>3200</td>
<td>0.007</td>
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<td>1.5</td>
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<td>200</td>
<td>0.015</td>
<td>8000</td>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td>The present work</td>
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<td>33000–200000</td>
<td>1.5</td>
<td>9000–1500</td>
<td>3</td>
<td>10–1</td>
</tr>
</tbody>
</table>

Table 1 Characteristic parameters for a number of selected existing micro-mixers: Reynolds number $Re$, Péclet number $Pe$, mixing volume $V_{mix}$, mixing time $T_{mix}$, lateral mixing dimension/mixing length $L_{mix}$, mixing time per volume of liquid $(T/V)_{mix}$.26 See Nguyen & Wu24 for a more complete overview of micro-mixers.
are integrated in a micro-fluidic channel. In this paper we show that our artificial cilia can generate substantial fluid flow, and that they can be used to mix fluids actively and effectively in micro-fluidic channels.

Results and discussion

Artificial cilia

Our artificial cilia are shown in Fig. 1. The typical structure is that of a curled micro-beam consisting of a double-layer of a thin polymer film, namely polyimide (PI) with a thickness of 1 μm, and a thin conductive chromium (Cr) layer with a thickness of 20 nm. The micro-actuators are made using micro-systems technology processing, as described in the next section. The radius of curvature of the micro-actuators is controlled by tuning the mechanical stress in the PI and Cr films by adjusting the deposition conditions and the relative thickness of both films. Note, that the artificial cilia are significantly larger than biological cilia that have a typical length of 10–20 μm. The technology used allows the miniaturization of the actuators by an order of magnitude, but for the purpose of a proof-of-concept demonstration we show only results of the larger artificial cilia shown in Fig. 1.

The actuation of the artificial cilia is done electrostatically: by applying a voltage difference between the ITO electrode and the Cr layer, an electrostatic attraction force is induced and the curled micro-actuator rolls out and extends over the surface. After switching off the voltage, the structure rolls back to its original curled shape by elastic recovery. We used an AC voltage with a frequency of 1 kHz, that was switched on and off with switching frequencies between 1 and 200 Hz. The cilia did not react to the AC-frequency but they rolled out and rolled back following the switching frequency. A minimum voltage is required to actuate the artificial cilia, determined by the materials and geometry applied. The rolling-out threshold voltage was around 70 V for our micro-actuators. With the use of a high-speed camera, we determined the rolling-out and rolling-back times. Upon applying the AC voltage, the micro-actuators reacted almost instantaneously. Movie S1† shows the artificial cilia being actuated in air (ESI). In air, the rolling-out time was very fast, namely 7 μs, and the rolling-back time was measured to be about 20 μs. In a silicone oil with a viscosity of 9.3 mPa·s, these numbers were less than 1 ms, and about 3 ms, respectively. The rolling-out time turned out to depend on the magnitude of the applied voltage $V_m$, namely 1 ms for $V_m = 70$ V and 0.2 ms for $V_m = 140$ V. The rolling-back time, on the other hand, was essentially independent of the applied voltage, which is understandable because rolling-back is driven merely by elastic recovery.

We carried out durability tests by continuous actuation in air at a switching frequency of 1 Hz. Only after one week of actuation, the first micro-actuators started to fail, that is after about 600,000 switching cycles.

Particle tracking experiments

To investigate the effectiveness of fluid manipulation by the artificial cilia, we carried out particle tracking experiments in silicone oil, from which the induced flow velocities were estimated. The cilia were arranged on a substrate in square segments of 1 mm², as shown in a top view in Fig. 2(a). The segment contains five columns of twenty cilia, visible in the figure as black rectangles since they are in the curled state. The surface was covered with a 0.5 mm thick silicone oil film (viscosity 9.3 mPa·s), so that the cilia were completely immersed. To visualize the flow, we carried out various experiments using two kinds of tracer particles dispersed in the fluid, namely titanium dioxide (TiO₂) particles with a mean diameter of 0.5 μm and hollow glass spheres with an average diameter of 12 μm.

Fig. 2 shows the estimated flow speeds as a function of switching frequency and applied voltage. Flow speeds up to 0.6 mm·s⁻¹ were generated. The flow direction is determined by the rolling-out direction of the micro-actuators. The effect is clearly demonstrated by movie S2† (available in the ESI). The induced velocity increases with both the switching frequency and the actuation voltage, and the agreement between the two types of particles is reasonably good.

Clearly, the artificial cilia are very effective in producing fluid flow. This raises the question as to the basic mechanism causing the flow. In small systems such as the present one, it is expected that Stokes flow conditions prevail, which means that inertia does not play a significant role, and the flow is dominated by viscous effects. This is expressed by the Reynolds number being very small, i.e. $Re = \rho UL/\eta \ll 1$. The flow is then completely reversible in time, and in this situation the motion of the cilia must be asymmetric in order to have a net effect on the flow.
The bottom channel wall is thus covered with a 0.5 mm thick film of silicone oil (viscosity 9.3 mPa·s), containing either TiO₂ or hollow glass as tracer particles. The cilia were actuated with different switching frequencies and actuation voltages, and the movement of the tracer particles was recorded at 30 frames per second. Particle tracking was done manually from the obtained movies, and the induced flow velocities were estimated.

Having established the effectiveness of flow generation by our artificial cilia, we now investigate the details of flow generation and its dependence on various parameters. We designed and fabricated a Y-shaped mixing channel, shown in Fig. 3. A polydimethylsiloxane (PDMS) cover, containing the Y-shaped channel structure, is mounted on top of the glass substrate with the artificial cilia. The bottom channel wall is thus covered with a 0.5 mm thick film of silicone oil (viscosity 9.3 mPa·s), containing either TiO₂ or hollow glass as tracer particles. The cilia were actuated with different switching frequencies and actuation voltages, and the movement of the tracer particles was recorded at 30 frames per second. Particle tracking was done manually from the obtained movies, and the induced flow velocities were estimated.

Mixing experiments

Having established the effectiveness of flow generation by our artificial cilia in an open-faced channel, we tested the concept of mixing in a micro-fluidic channel. We designed and fabricated a Y-shaped mixing channel, shown in Fig. 3. A polydimethylsiloxane (PDMS) cover, containing the Y-shaped channel structure, is mounted on top of the glass substrate with the artificial cilia. The bottom channel wall is thus covered with a 0.5 mm thick film of silicone oil (viscosity 9.3 mPa·s), containing either TiO₂ or hollow glass as tracer particles. The cilia were actuated with different switching frequencies and actuation voltages, and the movement of the tracer particles was recorded at 30 frames per second. Particle tracking was done manually from the obtained movies, and the induced flow velocities were estimated.

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two counter-rotating transverse vortices with different sizes in a segment. In neighboring segments, the positions of the larger and the smaller vortex are interchanged. This leads to a transverse flow pattern which resembles that of the staggered herringbone mixer. In both designs, the basic idea is that mixing should occur by the repeated change in transverse flow as the fluid travels through the channel. Two consecutive segments make up one “cycle”. A sufficient number of cycles should be passed for complete mixing to occur. As mentioned before, for the staggered herringbone mixer, for example, this number is between four and ten cycles, depending on the flow conditions.

We carried out flow visualization experiments using silicone oil with two colors. The fluid entering through the two inlets was colored with a red and a blue dye, respectively. The main flow rate, driven by the external pumps, was varied between 10 μl min⁻¹ and 60 μl min⁻¹, corresponding to mean velocities between 0.33 mm s⁻¹ and 2 mm s⁻¹. The experiments were done with silicone oils of two viscosities, namely 9.3 mPa·s and 0.93 mPa·s. These conditions correspond to global Reynolds numbers between 0.03 and 2.

With the artificial cilia switched off, the two differently colored fluid streams remained separated and did not mix. This proved that the non-actuated cilia did not influence the flow and that diffusion was not important. After switching on the cilia, all measurements showed efficient mixing. Fig. 4 shows snapshots from a mixing visualization experiment with the low-viscosity silicone oil. The layout corresponds to the A–B–A design of Fig. 3. The externally driven flow is from left to right. The flow rate is 60 μl min⁻¹, which corresponds to a mean velocity of 2 mm s⁻¹ and Re = 2. For t < 0 s, the two separate colored fluid streams are clearly visible. At time t = 0 s, all artificial cilia were switched on with a frequency of 50 Hz at an AC voltage of 100 V/1 kHz. A meandering flow pattern occurred within 0.04 s, it evolved, and the fluid was observed to be homogeneously mixed within 1.5 s. During this time, the fluid traveled not even 1.5 cycles through the channel, which is significantly less than what is achieved by known passive groove-type mixers. Movie S3† (available in the ESI) shows a recording of the mixing.

Fig. 5 shows the mixing results for the higher-viscosity silicone oil, and the mixing pattern design C–D–C of Fig. 3, analogous to the mixing results shown in Fig. 4 for the lower viscosity fluid and design A–B–A. Now, the flow rate is 10 μl min⁻¹, which corresponds to a mean velocity of 0.33 mm s⁻¹ and Re = 0.03. Also in this case, a meandering flow pattern emerges after switching on the artificial cilia (at 50 Hz switching time, with an AC voltage of 100 V/1 kHz), although more slowly than in Fig. 4. After about 9 s, the fluids are reasonably homogenized. In terms of number of cycles traveled through the channel, this time corresponds to less than 1.5 cycles, just as for the experiment of Fig. 4. Again, this is faster than expected on the basis of passive groove-type mixers. Movie S4 shows a recording of the mixing.

The artificial cilia create very efficient mixing in the micro-channel. However, it seems that, for mixing to occur by our artificial cilia, it is not required that the fluid travels through the channel and passes through a multiple number of repetitive mixing cycles. On the contrary, mixing seems to happen quite rapidly.

![Fig. 4 Snapshots from a mixing experiment using dyed silicone oils (viscosity 0.93 mPa·s) and mixing configuration design A–B–A from Fig. 3. The externally driven main mean velocity (from left to right) is 2 mm s⁻¹. At time t = 0 s the artificial cilia are switched on with a frequency of 50 Hz at an AC voltage of 100 V/1 kHz. (a) Not actuated. (b) t = 0.00 s. (c) t = 0.04 s. (d) t = 0.08 s. (e) t = 0.12 s. (f) t = 0.16 s. (g) t = 0.44 s. (h) t = 1.44 s. A meandering flow pattern almost immediately occurs, and within 1.5 s, the fluid is homogeneously mixed. This time corresponds to less than 1.5 cycles traveling distance in the main flow direction. Movie S3† (ESI) shows a recording of this experiment.](https://example.com/fig4)
Snapshots from a mixing visualization experiment using dyed silicone oils, as in Fig. 4, but here, the viscosity is 9.3 mPa\cdot s and the mixing configuration is design C–D–C (Fig. 3). The externally driven mean velocity (from left to right) is 0.33 mm s\(^{-1}\). At time \(t = 0\) s the artificial cilia are switched on with a frequency of 50 Hz at an AC voltage of 100 V/1 kHz. (a) Not actuated. (b) \(t = 0.00\) s. (c) \(t = 0.24\) s. (d) \(t = 0.48\) s. (e) \(t = 0.72\) s. (f) \(t = 0.96\) s. (g) \(t = 2.68\) s. (h) \(t = 8.76\) s. Although more slowly than in Fig. 4, a meandering flow pattern emerges and the fluid is reasonably homogenized after about 9 s, which corresponds to about 1.5 cycles traveling distance, as for Fig. 4. Movie S4† shows a recording of the mixing.

locally over just one cycle. The reason may be that the cilia do not generate a single, homogeneous vortex with one rotation rate, but a more complex flow involving more than one length- and timescale. This idea is supported by examining the dimensionless Stokes number \(St = \frac{\rho L^2}{\eta t_c}\), in which \(t_c\) is a characteristic (instationary) timescale. The Stokes number represents the ratio of unsteady effects over viscous effects. If \(St \ll 1\), then unsteady effects can be neglected and the flow can be considered to be (quasi-) stationary. As a characteristic timescale \(t_c\), we choose the reciprocal switching frequency, which is 1/50 s in Fig. 4. As the characteristic length \(L\) we take the channel width, i.e. \(L = 1\) mm. A value of \(St = 50\) is then found for the low viscosity silicone oil used in Fig. 4. For the high viscosity oil (Fig. 5), the value is \(St = 5\). Both are significantly larger than one, indicating that unsteady effects can be substantial. This suggests that the flow generated by the artificial cilia is composed of a combination of a global vortex with a superposed secondary oscillating flow. We attribute the observed effective mixing to this combination of time- and length-scales.\(^{39}\)

The performance of our artificial cilia based micro-mixer can be compared to the existing micro-mixers that were briefly discussed in the introduction, and of which characteristic parameters are listed in Table 1. The table also contains the characteristic numbers for our micro-mixer. The most direct comparison may be made with the staggered herringbone groove mixer of Stroock et al.\(^{20}\) since the geometrical resemblance between our mixing channel and the herringbone mixer is largest.

Like the herringbone mixer, our concept has been shown to work in a wide range of Reynolds numbers, and at very high Pécelt numbers. Our mixing volume is larger, and our mixing times are within the range reported by Stroock et al.\(^{20}\) Since we need less mixing cycles (as mentioned before), our mixing length is smaller. The efficiency of the artificial cilia mixer, characterized by the mixing time per mixed volume \((T/V)_\text{mix,i}\), is in the lowest-value range measured for the herringbone mixer, that is in the most efficient range. An obvious advantage of the active artificial cilia concept over the passive herringbone mixer is that the mixing can be switched on or off.

Compared to the other passive mixing concepts listed in Table 1, the artificial cilia concept has several advantages, next to being actively controllable. Our concept has been shown to work at low Reynolds numbers (in contrast to the serpentine channel of Liu et al.\(^{18}\)), and at very high Pécelt numbers (in contrast to the branching channel of Bessoth et al.\(^{17}\)), and it mixes much larger volumes than the droplet mixing approach of Song et al.\(^{19}\)

In terms of \((T/V)_\text{mix}\), the active mixers shown in Table 1 perform, in general, worse than the passive mixers. Exceptions are our artificial cilia mixer and the electro-hydrodynamic mixer of El Moctar et al.\(^{23}\) which show a comparable efficiency. An advantage of our approach over the other mixers is that the proven working range of Reynolds numbers is broader, and that the mixing volume is larger. The mixing lengths are similar for all listed active mixers.

In summary, our artificial cilia mixing concept compares favorably with other passive and active micro-mixers and has...
a number of key advantages. On the downside, the fabrication of the artificial cilia is not simple and this adds to the total cost of producing a micro-fluidic device. Cheaper solutions like passive mixing may be preferred for applications that do not require active control. Also, in the electrostatic actuation concept presented in this paper, highly conductive working fluids may interfere with the operation of the device by screening of the charges present at the electrodes. Electrochemical effects like electrolysis, on the other hand, can be avoided by using higher frequency AC signals (like in our experiments). Instead of using electrostatic actuation, magnetic actuation of artificial cilia can be used as well, and this eliminates the problems associated with electrostatic actuation.  

Finally, the largest benefit of the micro-fluidic manipulation by artificial cilia may be its versatility. We have shown that we can generate both transportation and mixing flows. Designing a proper geometrical arrangement of artificial cilia, in combination with a patterned electrode structure that allows for position-dependent actuation schemes, it will be possible to generate a range of controlled flows, from transportation to mixing, with one and the same cilia-layout.

Fabrication and experimental methods

Manufacturing of the artificial cilia

The artificial cilia are manufactured using a number of process steps, of which three are illustrated in Fig. 6. The substrate is glass with a 70 nm layer of indium tin oxide (ITO). The ITO is structured, by wet etching, to obtain the desired electrode pattern. A 1 μm dielectric stack is subsequently deposited. The stack consists of a 400 nm silicon oxide film, a 200 nm silicon nitride film, and another 400 nm silicon oxide film (a so-called ONO-stack). These films are deposited by PECVD (plasma enhanced chemical vapor deposition). A 300 nm aluminium (Al) layer is sputtered on top of the dielectric layer (see Fig. 6), and subsequently patterned using PES etching. The Al layer will, in a later stage, be removed to free the micro-actuators, i.e. it is a sacrificial layer. On the patterned Al, a 20 nm chromium (Cr) layer is sputtered under conditions that lead to a high tensile stress in the Cr. After a UV–ozone surface treatment, a polyimide (PI, Durimide 9005, manufacturer Arch Chemicals Inc.) film is spin-coated on the Cr with a rotation speed of 850 rpm (see Fig. 6). The PI is photo-sensitive and can thus be directly patterned in the shape of the micro-actuators using lithography. First, a hotplate prebake at 120 °C is applied for 3 min. The PI is then illuminated through a mask using UV (15 s at 9.3 mW cm⁻²) and developed (TMA238WA developer + H₂O, 50 s). After rinsing with water, the patterned layer is dried (5 min at 110 °C) and cured (1 h at 350 °C). The Cr is then etched (using cerium ammonium nitrate) at the places where it is uncovered by PI, i.e. the PI acts as an etching mask (see Fig. 6). The last step in the process is to etch the Al films from underneath the micro-beam patterns using PES etching, which leaves the Cr unaffected. During this process step, the artificial cilia are released from the surface, and curl upwards due to the internal stress present in the structure that is caused by the different properties and thicknesses of the PI and the Cr layers.

Manufacturing of the mixing channel

The Y-shaped mixing channel shown in Fig. 3 was fabricated as follows. The artificial cilia were manufactured on a glass substrate as described earlier. A PDMS cover, containing the Y-shaped channel structure, was mounted on top of the glass substrate. The PDMS cap was made from a mixture of 10 : 1 silicone elastomer base and curing agent (Sylgard 184, Dow Corning). The degassed liquid was poured into a mould and cured in an oven at 80 °C for at least 6 h. To attach the PDMS to the glass substrate, an O₂ plasma treatment (0.48 mbar, 300 W, 1 min) was applied to the PDMS, after which the PDMS cap was manually adjusted onto the glass substrate. To avoid the swelling of the PDMS by the silicone oil (working fluid), the activated PDMS was, immediately after attachment to the substrate, modified with a perfluorinated monolayer (1H,1H,2H,2H-perfluorodecyltrichlorosilane; ABCR GmbH), using gas-phase deposition in vacuum for one hour.

Driving the artificial cilia

The actuation of our artificial cilia is done electrostatically by applying an (AC) voltage difference between the ITO electrode and the Cr layer. We used an AC voltage with a frequency of 1 kHz, that was switched on and off with typical switching frequencies between 1 and 200 Hz. The electrical signal was generated by a waveform generator (Agilent, 33220A) in combination with an amplifier (Kroh-Hite corporation, 7602M). In the experiments reported here, all artificial cilia were addressed simultaneously.

High-speed camera measurements

To determine the speed of the rolling-out and rolling-back of the artificial cilia both in air and in silicone oil we used a high speed camera (a Princeton intensified camera, PI-MAX (chip THM 512 × 512) with a ST133 controller) mounted on an optical microscope. We focused on the cilia, obtaining a top view as in Fig. 2(a), but with a higher magnification, such that only 2
or 3 cilia were visible in the field-of-view. During a rolling-out and rolling-back event, images were recorded at a frame time of 25 µs in silicone oil and 500 ns in air. These images were analyzed using a MatLab-based software routine to obtain the rolling times.

**Particle tracking measurements**

We carried out particle tracking experiments, of which the results are shown in Fig. 2. The cilia were arranged on the surface in square segments of 1 mm², as shown in a top view in Fig. 2(a). The segment contains five columns of twenty cilia, visible in the figure as black rectangles since they are in the curled state. The surface was covered with a 0.5 mm thick silicone oil film (Wacker AK10 silicone oil, viscosity 9.3 mPa·s and density 930 kg m⁻³), so that the cilia were completely immersed. Two kinds of particles, dispersed in the fluid, were used to visualize the flow, namely titanium dioxide (TiO₂) particles with a mean diameter of 0.5 µm and hollow glass spheres with an average diameter of 12 µm. The former have a density of 4.5·10³ kg m⁻³, and the latter were matched to the density of the silicone oil, i.e. 930 kg m⁻³. Other types of particle were tried as well: silica microspheres, polystyrene, kaolinite. All of these, however, responded substantially to the electrical field, applied to actuate the artificial cilia, by electrophoresis and/or dielectrophoresis, and they were therefore unsuitable for studying the fluid flow velocity since their movement would not represent the actual fluid movement. This effect was minimal for the used TiO₂ and hollow glass particles. Observations were done using an optical microscope. After switching on the actuation voltage, images were taken at 30 frames per second, particle tracking was done manually from the obtained movies, and the induced flow velocities were estimated.

**Mixing visualization experiments**

Mixing visualization experiments were carried out using the Y-shaped mixing channel device described earlier. Two different silicone oils were used in different experiments, i.e. Wacker AK10 silicone oil, with a viscosity of 9.3 mPa·s and a density of 930 kg m⁻³, and Wacker AK1 silicone oil, with a viscosity of 0.93 mPa·s and a density of 930 kg m⁻³. The fluid, entering through the two inlets, was colored with a red and a blue dye, respectively, namely Oil Red EGN and Oil Blue N (both Sigma-Aldrich). The dies (in powder form) were added to the silicone oil up to the saturation value; the mixture was subsequently heated at 80 °C for 5 min and filtered using a teflon filter with a pore size of 0.45 µm. The segment contains five columns of twenty cilia, visible in the figure as black rectangles since they are in the curled state. The fluid, entering through the inlets, with 2 ml plastic syringes (BD Discardit II). The flow was varied between 10 µl min⁻¹ and 6 µl min⁻¹ for the higher-viscosity fluid, corresponding to mean velocities between 0.33 mm s⁻¹ and 0.2 mm s⁻¹. For the low-viscosity fluid, a flow rate of 60 µl min⁻¹ or mean velocity of 2 mm s⁻¹ was used. The flow was observed with an optical microscope (Leica MZ6), and images and movies were recorded with a color camera (CV-S2500, Jai corporation, Japan) in combination with image analysis software (Pinnacle Studio, version 9.02, Pinnacle Systems).

**Conclusion**

We have demonstrated that our integrated artificial cilia can generate substantially high flow velocities in open-faced micro-channels, as well as efficient mixing flows in micro-fluidic channels. The concept, therefore, appears to be quite versatile. The mixing efficiency compares favorably with other passive and active micro-mixers and has a number of key advantages. The micro-mixing concept using artificial cilia has been proven to work in a wide range of Reynolds numbers, and at very high Péclet numbers. We have shown that the mixing length is shorter than for known passive mixing configurations such as the herringbone mixer. The mixing efficiency, expressed in mixing time per volume of mixed liquid, is better than for existing active micro-mixers. With the use of patterned electrodes, the effect can be switched on or off on demand, at locations of choice. These properties make artificial cilia attractive and promising for future integrated micro-fluidic devices in which active fluidic control is required, particularly in lab-on-a-chip devices for (bio-)chemical analysis in which reagents are added or stored either in a liquid or a dry state, and must be mixed quickly in micro-reaction chambers.

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**References**

12. The Reynolds number is defined as \( Re = \frac{UL}{\nu} \), where \( U \) is a characteristic fluid velocity for known passive mixing configurations such as the herringbone mixer. The mixing efficiency, expressed in mixing time per volume of mixed liquid, is better than for existing active micro-mixers. With the use of patterned electrodes, the effect can be switched on or off on demand, at locations of choice. These properties make artificial cilia attractive and promising for future integrated micro-fluidic devices in which active fluidic control is required, particularly in lab-on-a-chip devices for (bio-)chemical analysis in which reagents are added or stored either in a liquid or a dry state, and must be mixed quickly in micro-reaction chambers.

13. The Péclet number is defined as \( Pe = \frac{UL}{D} \), in which \( U \) is the characteristic flow velocity, \( L \) is the characteristic length scale of the flow (for example the channel width), and \( \nu \) and \( \eta \) are the density and the dynamic viscosity of the fluid. In channel flow, turbulence may occur for Reynolds numbers larger than about 2300, however in micro-fluidic systems Reynolds numbers are usually much lower, i.e. order one or less.

26 Note, that we report the typical Re, Pe and other parameter values as they have been reported in the literature published on the specific micro-mixers. Some concepts may be scalable and work just as well in other ranges of the parameters.

27 Cilia are structurally identical to flagella, and the terms are often used interchangeably. In general “cilia” is used when the rods are numerous, short, and co-ordinated, while “flagella” is used when they are sparse and long.


35 Applying a similar analysis to the natural cilia shows, that the local Reynolds numbers are much lower in that case (namely much smaller than one) and inertia is not playing any role.

36 The definition of what “complete mixing” is, is not straightforward. One could say that a completely mixed system consists of a combination of two or more substances that are homogeneously distributed over the whole system, down to the molecular scale. For practical applications, it is often sufficient to reach a certain level of homogenization that is coarser, and that can be quantified using various measures for mixing. In this paper, we look at mixing only qualitatively, based on observations of microscopic images. A clear discussion of the subject is given in: C. L. Tucker III, in Mixing in Polymer Processing, ed. C. Rauwendaal, M. Dekker, 1991, pp. 101–127.

37 The diffusion coefficient of the dyes in the silicone oil with a viscosity of $\eta = 9.3 \text{ mPa}\cdot\text{s}$ can be estimated as $D \approx 1 \times 10^{-11} \text{ m}^2\text{s}^{-1}$, in the silicone oil with $\eta = 0.93 \text{ mPa}\cdot\text{s}$ this is $D \approx 1 \times 10^{-10} \text{ m}^2\text{s}^{-1}$. For $L$ we take the channel width, and for $U$ the mean axial velocity. This gives Péclet numbers in the range of 20000 to 33000. Clearly, diffusion is not expected to play any role.

38 Note that mixing times may be somewhat underestimated since we observe the flow evolution from the top and see an effect that is integrated over the channel thickness, instead of imaging a cross section, see: C. Xi, D. L. Marks, D. S. Parikh, L. Raskin and S. A. Boppart, Proc. Natl. Acad. Sci. U. S. A., 2004, 101, 7516–7521.

39 In fact, the mixing efficiency in turbulent flows is due to the existence of a broad, continuous range of length- and time-scales within the flow. Although not turbulent, the mixing in our mixing channel experiments may be caused by a similar generation of multiple length- and time-scales.