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AN INTEGRATED SYSTEM WITH MAGNETICALLY DRIVEN CILIA

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KEY WORDS

Artificial cilia, polymer composites, magnetic actuation system, lab on a chip

ABSTRACT

It has been shown theoretically that flow [1] and fluid mixing [2] [3] of volumes of a few micro liters can be realized by magnetically actuated artificial cilia. In this paper, we describe an experimental integrated system in which magnetically actuated artificial cilia could generate flow through microchannels. Our approach enables the integration of the magnetic cilia arrays in microfluidic devices in the category of lab on a chip. The system consist of a microfluidic cartridge in which the artificial cilia are integrated, and a magnetic actuation system to generate the magnetic field that controls the cilia movement. The cartridge allows optical access to enable observation of flow, cilia movement, and possible other processes occurring within the cartridge. A micro-prism integrated in the cartridge makes easy side-view observation possible. For the magnetic actuation system, a quadruple design is adopted with the pole tips optimized by simulations with a finite element method. In this way the balance between the field strength and uniformity is obtained for cilia arrays up to 10 millimeters in length. Alternatively, a rotating permanent magnet can be used for actuation. To illustrate the possibilities of the system, we present an analysis of the movement of the magnetic cilia within the cartridge.

1. INTRODUCTION

Fluid manipulation in microchannels is required in a variety of applications, such as in miniaturized systems for bio-molecular sensing, drug delivery and cell analysis. For these applications, the use of so called "lab on a chip" cartridges makes it possible to perform the same operations as in an advanced bio-chemical laboratory, but miniaturized, highly integrated, with better performance, and highly cost-effective. Currently, the common methods to achieve fluid manipulation are based on down scaling macroscopic flow devices such as pumps, valves and mixers or using physical principles that are advantageous at small scales like surface tension, surface energy pattering, electro-osmosis etc. In this paper we address an alternative method of fluid manipulation inspired by nature, namely by the actuation of artificial cilia. One can simply describe a cilium as a small hair, tens to hundreds of microns long and attached to a surface. Beating of one cilium or synchronously moving *natural* cilia arrays enables the swimming of micro-organisms like paramecium. *Our objective is to create an integrated system in which magnetically actuated artificial cilia generate flow through microchannels.*The integrated system consists of two parts: a cartridge with integrated artificial cilia arrays and a magnetic actuation system. The system is designed such that optical access of the cartridge is possible to allow characterization of processes occurring within the cartridge such as biochemical reactions, flow, or cilia dynamics.

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This paper describes the system. We present first the composite materials used for the artificial cilia as well as a summary of the fabrication methods used. This is followed by a section in which the cartridge, in which the artificial cilia are integrated, is described. In the last experimental part we present the magnetic actuation systems designed for the cilia magnetic actuation. Finally, we present some selected results achieved with the system.

2. MATERIALS AND CILIA ARRAY FABRICATION

Our artificial cilia consist of polymer films in which magnetic nano-particles are dispersed, and that are structured in the shape of small flaps anchored to the surface. For the polymer matrix material, two different classes of materials were used. One class is based on poly(N,N-dimethylacrylamide) PDMAA hydrogel that gains its elasticity by swelling in aqueous media. The second class is a rubber material based on poly (n-butylacrylate) (PBA). Both materials were synthesized such that they contained a few percent of a photocrosslinkable monomer. They can be deposited on surfaces via conventional coating techniques and it has been shown that spin casting is the most feasible way to generate films of a few hundred nanometres in thickness. Upon irradiation with UV light the films crosslink and become insoluble in common solvents.

The magnetic nano-particles dispersed in these films are colloidal particle systems that were provided by Liquids Research Limited, UK. Two different systems were used. One was based on superparamagnetic (SPM) particles of Fe_3O_4 and one was based on remanent magnetic (RM) particles of Cobalt-ferrite. Both particle systems could be used in colloidal state when dispersed in the polymer solution. Using SPM particle systems for the cilia is specifically advantageous if magnetic beads are being used in the biochemical assay that needs to be performed in the device. Magnetic beads are often used to capture the targeted molecules (e.g. proteins in immune assays) from the working fluid. Since the SPM particles are not magnetized in the absence of an external magnetic field, no magnetic interactions between the artificial cilia and the beads are expected when the magnetic field is removed. However the use of SPM particles has the disadvantage of low magnetization per cilia therefore relatively large external magnetic fields up to 30mT are required for actuation. If the requirement of magnetic interactions between the cilia and the fluid particles does not apply, then using composite materials with RM particle has the advantage of requiring much lower fields for actuation.

Due to the compatibility of the developed surfactants that cover the magnetic nano-particles, it was possible to integrate both the SPM system and the RM system in our polymer films to create the composite materials for the cilia. According to an analysis with XPS and TGA, the particles are homogenously distributed within the hydrogel matrix and have an increasing density from the top to the bottom for the rubber matrix. Both types of structures were actuated in magnetic fields.

Furthermore, an induced anisotropy field H_i was obtained for the composite materials containing Cobalt ferrite particles having an average size of 10nm, see Figure 1a. In this case the composite material was fabricated under magnetic field. According to our TGA analysis the films were loaded with 38%w magnetic particles. The magnitude of H_i can be tailored depending on the applied field used during the fabrication procedure. At 1T uniform field applied during the synthesis we obtained $H_i \approx 100$Oe. Optical inspection of the composite layers revealed that the sub-micrometer aggregates are aligned on columns parallel to the applied field, see Figure 1b. By controlling the induced anisotropy of the artificial cilia materials we have the possibility to fully control the orientation of the magnetic force during the actuation process [4]

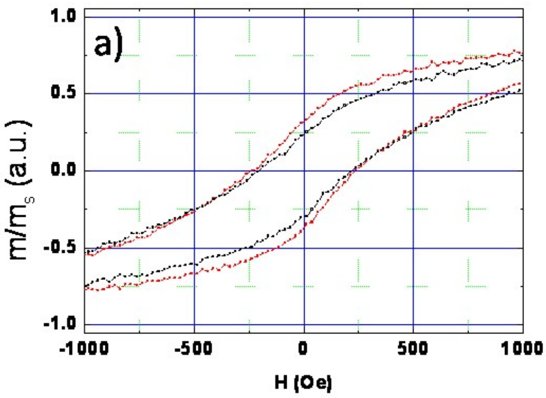
The details of the fabrication process of structuring the composite films into artificial cilia are given in [5] and [6]. In this paper we mention only the most important aspects. The fabrication process of the artificial cilia consists of the following steps:

1) Functionalization of the Si-wafer with an alkylsilane monolayer
2) Spin coat a sacrificial layer, which is a polymer film that can be UV-cured at a specific wavelength of λ=250 nm.
3) Pattern the sacrificial layer at λ=250 nm, to form the anchors with which the cilia will be attached to the surface.
4) Deposit the composite layer containing the magnetic particles and that will eventually form the artificial cilia
5) Pattern the composite layer at λ=365 nm; this leaves the uncured parts of the sacrificial layer unaffected.
6) Develop the composite layer; also this step leaves the sacrificial layer unaffected.

The removal of the sacrificial layer will take place after the cilia array is integrated in the micro-fluidic cartridge and the release fluid is introduced. During that step, the free-standing artificial cilia are created right before they have to be used. It is clear that special attention had to be given to the development of the composite material containing the magnetic particles and the sacrificial layer that had to be compatible to the artificial cilia procedure.

An azide based crosslinker was used initially in the fabrication of the anchor/ sacrificial layer. That turned out to be sensitive to heat. Samples stored for longer periods at elevated temperatures suffered from thermal crosslinking of the sacrificial layer. In these cases, a reliable release was not guaranteed. A non thermo-sensitive crosslinker based on ketone was designed, synthesized and successfully tested in the rubber system. Samples with the new crosslinker can be stored without any special arrangements and still work well after weeks.

Our final artificial cilia had a length of 80µm, a width of 20µm, and a thickness up to 500nm.



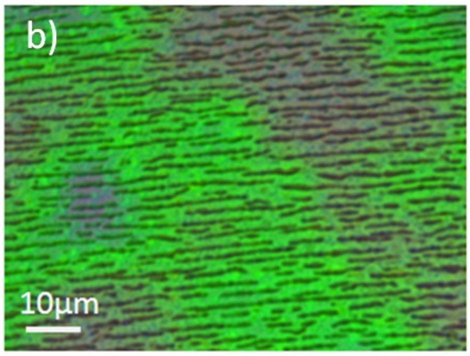


Figure 1 a) Hysteresis loops measured in plane of the Co-ferrite composite films with the external filed oriented parallel and perpendicular to the induced anisotropy axis; b) Bright field light microscopy on a composite film containing the cobalt ferrite particles; sample fabricated in a uniform and homogenous applied field of 1T.

3. MICROFLUIDIC CARTRIDGE

The artificial cilia were integrated in a microfluidic cartridge. We adopted two simple concepts, allowing for a proof-of-concept of the actuation and flow generating potential of our artificial cilia, both depicted in Figure 2.

a) One structure of our choice consisted of a single channel with one inlet and one outlet. In that case the artificial cilia orientation is along the channel. A sketch of this idea is presented in Figure 2a. The flow generation capacity of the artificial cilia can be determined by measuring the flow rate, or by determining the developed pressure head, or by direct visualization / quantification of the flow velocity using particle tracking or micro-PIV (Particle Image Velocimetry). In particular for the latter approach, the cartridge must be optically transparent. Note that the magnetic actuation system is not included in the image; this will be shown in the next section.

b) If the particles used as tracers in the microPIV setup have a long sedimentation time compared to the duration of a measurement, then one can use a return channel in the cartridge to quantify the flow velocities generated by the cilia within the channel, see Figure 2b. In that case the channel forms a closed loop and we have a direct measurement of the flow induced by the cilia array. Note, that the cilia are present only in one branch of the loop and are there oriented in the direction of the channel.

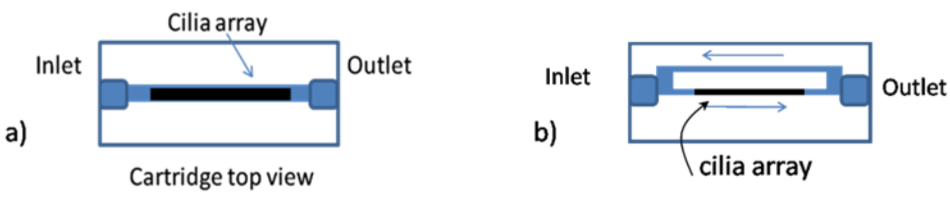


Figure 2 The two basic microfluidic cartridge concepts used: a) a single straight channel, b) a closed loop channel.

Apart from the basic experiments mentioned above, the cartridges can be used with a background flow generated with the aid of an external pump. Depending on the cilia orientation angle with respect to the background flow a fluidic mixing configuration can be obtained. Note that in this case the magnetic actuation conditions have to be adapted.

The cartridges were designed according to a laminated structure. A fully assembled cartridge consists of an optical part fabricated of polycarbonate or glass material containing the fluidic connections, a polydimethylslioxane (PDMS, silicone rubber) layer that defines the microfluidic channel and the artificial cilia array chip carrier. These three parts are stacked onto each other and in order to ensure that all three parts of the cartridge are kept as one unit, an additional outer channel is defined in the PDMS layer and optical part. In this channel vacuum is applied to hold all three parts together.

In Figure 3a we present a fully assembled single straight channel cartridge with a polycarbonate optical part. In the same image one can see the Si chip carrier with cilia arrays and the microfluidic channel as defined by the PDMS layer. A version of the return loop cartridge fabricated in glass is given in Figure 3b. Note that this design was made such that loading the cartridge with the fluid can be performed so that no air bubbles are introduced when the system is being filled with liquid. We have also observed that in the case of glass optical parts, the vacuum connection can be skipped due to the good adhesion of PDMS middle layer and the stiffness of both the substrate and the top part.

From the point of view of optical access, both cartridges can be used well up to objectives with 20x magnification. Further increase of magnification is possible with the glass optical part due to the fact that its window has a thickness of a microscope slide i.e. 0.7mm.

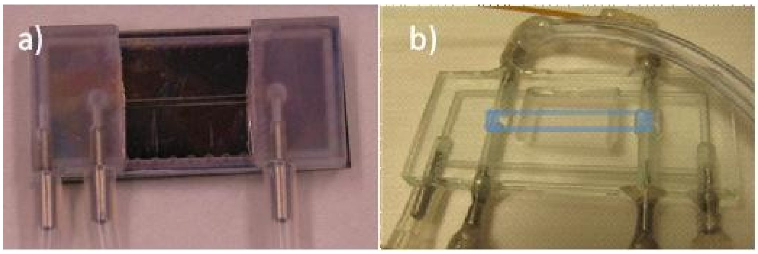


Figure 3 Fabricated cartridges for the integration of the artificial cilia arrays. a) The single channel configuration made with a polycarbonate optical part. b) The closed loop channel configuration made with a glass optical part, including a thin glass

To enable a full observation of cilia driven flow velocities, the experimental microfluidic cartridge can be expanded with the possibility to optically inspect the fluidic channel not only from the top but also from the side. By combining top and side view inspection, not only a more complete characterization of the flow can be achieved, but also the dynamic movement of the artificial cilia can be observed. For this purpose, a 45^o angle micro prism of 0.5 mm size and Al coated hypotenuse was integrated into the cartridge device at a location alongside the fluidic channel, see Figure 4a and b. Images with tracer particles as used in micro-PIV are presented in Figure 4c and d. In this way we demonstrate that the illumination conditions are sufficient for measurements of the velocity field in the vertical plane of the fluidic channel.

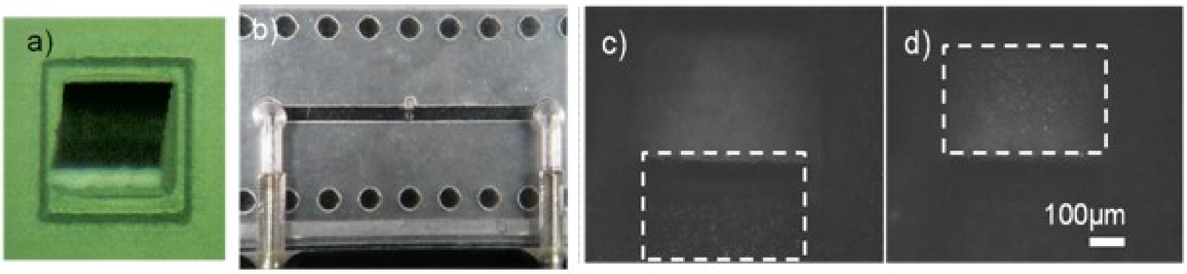


Figure 4 a) Micro-prism coated with reflective metallization (size 0.5 mm); b) optical part of the cartridge with integrated micro-prism (the PDMS layer was cut to allow for the space of the micro-prism and placed on the optical part); c) tracer particles as observed with the focus conditions on top of the microfluidic channel; d) tracer particles as observed with the focus conditions on the micro-prism.

4. MAGNETIC ACTUATION SYSTEM

For applications in lab on a chip area, magnetic actuation has advantages over most of the other actuation methods. The first important property is that the interaction between applied magnetic fields and biological fluids is so small that it can be neglected in most cases. Second, the plastic devices carrying the biological fluids are basically non-interacting with the applied magnetic fields so no disturbances or unpredicted field gradients can be formed. In this way the integration of the magnetic structures can be made easier as compared to the situation when integrated conductive structures are used for fluid, micro-actuators, or particle manipulation such as in electrostatic actuation as in Toonder et al. for electrostatic cilia [3]. In addition, the lab on chip cartridges that are used in conjunction to magnetic actuation can be designed such that no tubes for pneumatic control (used often for pressure-controlled micro-fluidic membrane valves and pumps) or electrical wires are attached. This offers substantial ease of use in practice, in which the processes occurring in the cartridge will mostly be controlled by an instrument in which the cartridge is inserted, since no physical connections need to be established between the instrument and the cartridge.

For our magnetic cilia, various actuation principles can be used depending on the nature of the magnetic particles distributed in the polymer matrix and the shape of the composite structures. If the microstructures to be actuated contain superparamagnetic particles, then we need to create a magnetic field gradient. Alternatively, if the magnetic field is uniform then the shape of the structures should be such that the internal magnetic field induced within the structures will be not uniform. Then, different parts of the structure will experience a different local torque due to the local variation of the internal field, which makes actuation possible. For the composite materials with remanent magnetic particles on the other hand, the actuation can take place using either an uniform or a spatially varying magnetic field regardless the shape of the structures.

To give an impression of the quantities involved, we give the expressions for the magnetic force $\vec{F}$ acting on a point-like magnetic dipole: $\vec{F} = (\vec{m}\nabla)\vec{B}$ where $\vec{m}$ is the magnetic moment and $\vec{B}$ is the magnetic flux applied. For superparamagnetic structures $\vec{m}_{spm} = \frac{V_p(\chi_p - \chi_f)\vec{B}}{\mu_0}$ where V_p is the volume and $\chi_{p,f}$ are the magnetic susceptibilities of the structures and fluid. For remanent particle systems, $\vec{m}$ can be considered approximately the same as the measured remanent value of magnetization. The approximation is valid as long as the actuation fields are small compared to the saturation field value. It is clear that having materials with high susceptibility, as well as being able to produce sufficiently high actuation fields are the most important features for controlling the actuation process. If we consider the cilia made with RM particles, the following typical parameter values can be used to estimate the required uniform magnetic field: remanent magnetization M_r=15kA/m, length L=80μm, width W=20μm, thickness T=0.5μm, uniformly distributed maximum magnetic force F_{max}=3nN. That would lead to a field induction $B=F_{max}L/M_rV=20mT$. The necessity of such field values was investigated in simulations presented elsewhere [1]. In designing the magnetic actuation system, our goal was the ability to generate magnetic field inductions of at least 25mT, achieving a high field uniformity at the magnetic cilia locations (no more than 5% variation over the cilia length), and the ability to control the magnitude and orientation of the magnetic field in time.

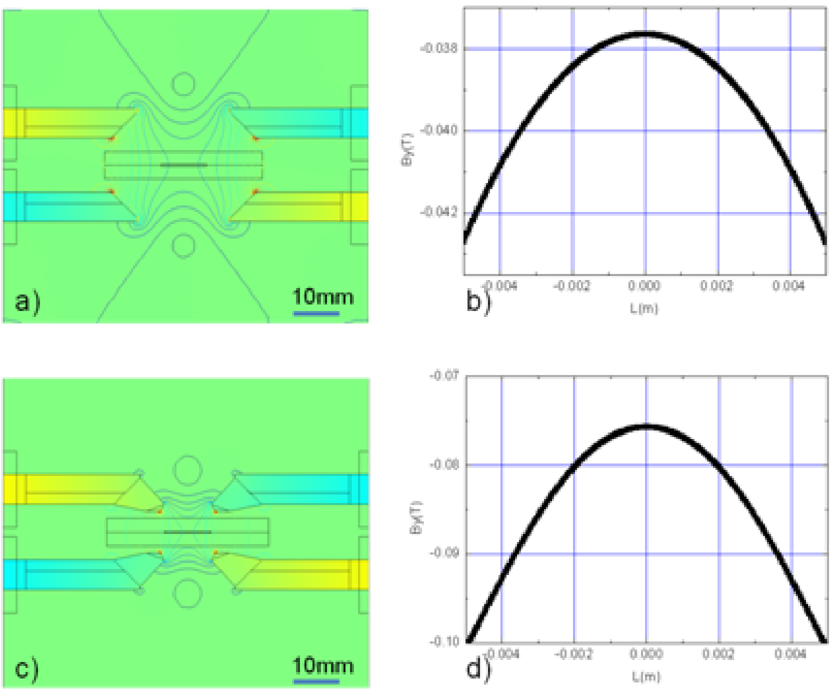


Figure 5 Simulations of the electromagnet magnetic field distribution. a) and c) normalized contour of magnetic field with different pole configurations; b) and d) calculated values of the magnetic induction By along the cilia array, corresponding to configurations a) and c) respectively.

A natural choice was an electromagnetic actuation system with four poles, between which the cartridge containing the magnetic cilia (described in the previous section) is placed, as sketched in Figure 5a) and c). To design the system, we simulated the field distribution in the space containing the cartridge using the commercial finite difference code COMSOL. Due to the symmetry of the problem in the vertical plane, we performed 2D simulations. Results of the simulations for the field oriented along the cilia array in the channel's length direction are presented in Figure 5. The input parameters were the current density J = 1A/mm^2, total coil length of 25cm and a permeability of the core material μ_r=500. In practice, higher current density values would require cooling of the electromagnet coils [7]. We tested two designs of the electromagnet poles. In the first geometry the poles have facets at an angle of 45^o giving an average field of 40mT with a maximum field variation of approximately 10% along the complete channel which is 10mm long, see Figure 5 a and b). The second poles geometry, depicted in Figure 5 c), leads to a even higher average field value of about 85mT but at the expense of an increased field variation of 20%. However in our experiments the cilia length is no longer than 100µm and therefore we can consider the field to be homogenous and uniform over the entire cilia length.

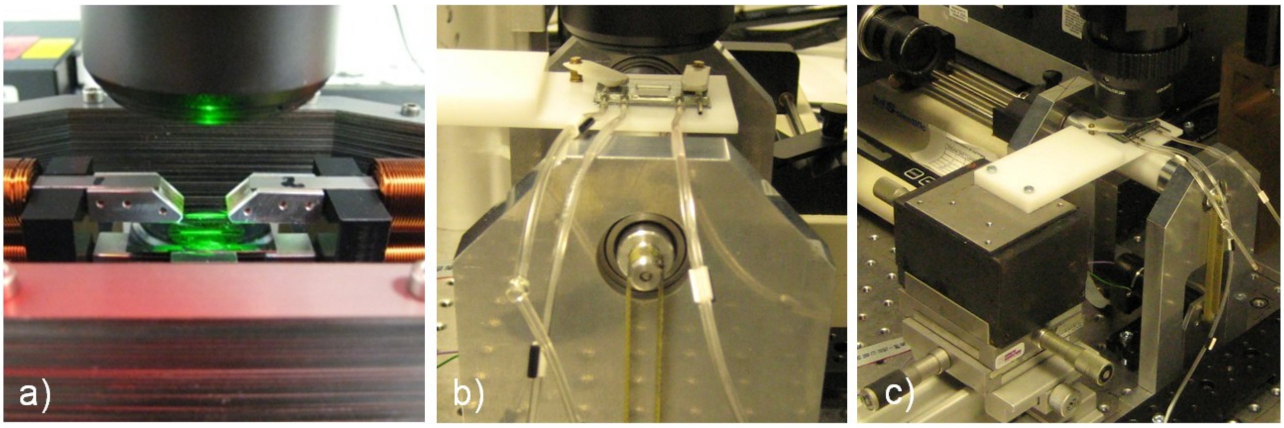


Figure 6 a) Electromagnet with laminated core; a microfluidic cartridge is placed between the poles and a microscope objective for optical characterization is visible above the set-up; b) permanent magnet set-up lateral view of the shaft connected with a belt to the electromotor; c) permanent magnet setup view with three axis alignment sample stage.

On the basis of these simulations, an electromagnet was fabricated that is shown in Figure 6a. It is noticeable that the magnetic core and the pole tips (of which only two out of four are visible in this image) are made of laminated material. The reason for choosing that was to avoid eddy currents if fast rise times for the currents were necessary for actuation. When a magnetic metal with a resistivity ρ=1x10^{-7}Ωm and a relative permeability μ_r=500 is magnetized by a 50Hz AC magnetic field, the skin depth is calculated to be $d_s = \sqrt{\frac{2\rho}{\omega\mu}} \approx 1mm$. The material used for the core was a Fe-Si alloy and it was laser cut. No thermal treatment was applied prior assembly. As a consequence we expect that the permeability values decreased due to cutting and stress due to compression of layers. Measurements showed that we could generate maximum values of 45mT when operating the system in DC mode.

Based on our theoretical investigations published earlier [1], we have identified two possible protocols for artificial cilia actuation when applying uniform fields. For the structures having remanent particles the actuation procedure is relatively simple: a field of certain strength will be placed at a certain angle (for instance 225^0) with respect to the rest position of the cilia for a certain time. The cilia will attempt to align with this field. Then, the field is reduced to zero for a given period of time. During this second step, the recovery of the cilia to their original shape will take place. This switching on and off of the field is periodically repeated. The kinetics of artificial cilia actuated in this way is characterized by a buckling type of instability that causes the cilia to beat in an asymmetric fashion.

For the flaps with superparamagnetic particles the actuation procedure consists of applying a field of constant magnitude that first rotates from 0 to 180^0 for a certain fraction of a period and then its angle stays constant. Here the key element in obtaining the asymmetry in movement of the cilia is the fact that at the end of the effective stroke, opposite local torques act upon the artificial cilia leading to strong deformation.

A software interface controls and stores information according to the nature of the artificial cilia used. The experimental setup consists of: two operational amplifiers used to power each coil pair of the electromagnet, a high quantum efficiency CCD camera suited for fast data acquisition, a laser or other high power light source and a NIDAq card with ADC and DAC functions. The NIDAq card is used to provide arbitrary input functions for the power amplifiers, to monitor the current through each pair of coils and to provide a trigger signal for the camera and light source. In this way synchronous measurements can be taken for example for a microPIV or flow visualization analysis.

Next to the electromagnetic system, a much simpler approach was used. We made use of only one neodymium boron magnet wide enough to produce fields able to align all flaps in the same direction when the magnet is oriented parallel to the cartridge plane. This magnet was attached to a shaft mechanically coupled to a small electromotor. The cartridge with the microfluidic channel is placed above the magnet at a distance of 1.5cm. The magnet can reach rotation speeds is 40 and 2000 rpm corresponding to frequencies of 1.3 to 66Hz. Note that the rotating shaft can be seen in Figure 6c under the sample stage.

The use of the permanent magnet set-up has the advantage of making possible cilia actuation at frequencies higher than the ones that can be produced with the electromagnet, since the latter are limited by the inductance values of the coils. Although more complex rotating permanent magnets systems can be fabricated for inducing uniform fields [8] we have chosen the one presented here due to reduced degree of vibrations.

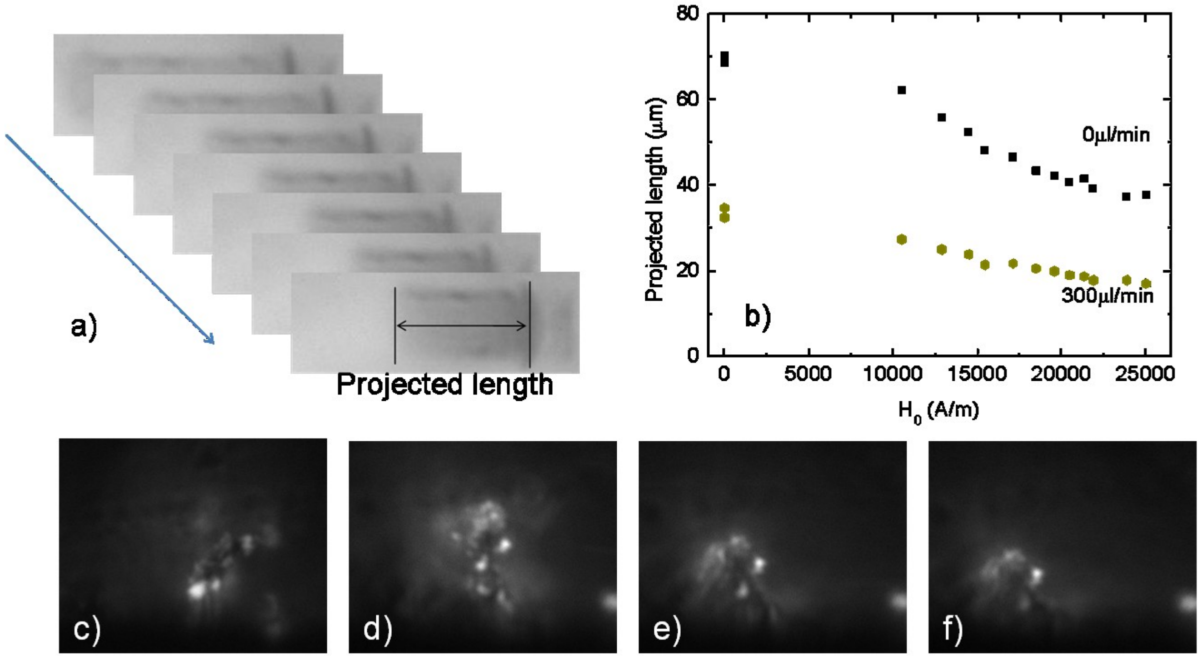


Figure 7 a) images of a cilium with increasing magnetic field applied in the vertical plane and zero background flow; the width of the cilium is 20µm; b) projected length as a function of applied field in zero and 300µL/min background flow; c) side view image of a cilium covered with fluorescent particles; d & e & f) the same cilia measured in a background flow of 300µl/min at magnetic field values of 0A/m, 12kA/m and 24kA/m

4. SELECTED RESULTS

In this paragraph we present a selected set of experimental data in order to illustrate the potential of the system with the integrated artificial cilia array. The cartridge is connected to a pump that delivers a background flow such that the cilia are lifted off the bottom in the absence of magnetic field. Measurements are performed with increasing the magnetic field from 0A/m to 25kA/m while keeping the field orientation in the vertical plane. In Figure 7a we present a sequence of top-view images of a cilium in zero background flow while applying a magnetic field as mentioned above. As expected, the projected length P_L of the cilium decreases with increasing the magnetic field. P_L values as a function of applied magnetic field are presented in Figure 7 b for 0 and for 300µl/min background flow . One can see that the deflection of the cilium in almost the same comparing the P_L measured in zero magnetic field and high background flow with the values for the zero flow and maximum magnetic field. In the first situation the drag force acting on the cilium is balanced only by the elastic force and in the second case the same elastic force is balanced only by the magnetic force. Therefore one can make an first estimate of the magnitude of the forces involved in these extreme conditions if we estimate the drag force F_{drag} acting on the cilium while applying only a background flow U=300µl/min which corresponds to an average fluid velocity in $v \approx 10x10^{-3} m/s$ for a channel height $h_{ch}=500\mu m$ and a channel width W_{ch}=1mm. We obtain in a first approximation $F_{drag}=6\pi\eta v S^{1/2} \approx$7nN, where S is the projected surface of the channel along the flow direction and the viscosity $\eta=1mPa\ s$.

In Figure 7 c-f) we present the side view image of a cilium obtained in a cartridge shown in section 3. The channel was filled with fluorescent particles prior the measurement. The visualization was possible due to the fact that some of the particles were adsorbed on the cilium. Note that in this case the cilium had a curved shape after the release procedure due to its hydrophobicity. In this way we can investigate the bending behavior of the cilium while changing the field magnitude and orientation for different background flow rates. A comparison can be made between the image in Figure 7 c and d where the same cilia is subjected or not to a 300µl/min flow from the right direction. In addition the cilia is presented when an external uniform field is applied in the plane of the cartridge, see Figure 7 e and f.

5. SUMMARY

In this paper we have presented an integrated system in which magnetically actuated artificial cilia generate flow through microchannels, and that allows optical characterization of the flow and the cilia movement. Although we have used it for proof-of-concept experiments, the same approach can be taken for a real (lab on a chip) device using cilia-driven flow. The system consists of two components: a microfluidic cartridge with integrated magnetic cilia, and a magnetic actuation system.

Our magnetic cilia are made of structured composite thin films consisting of a rubber or a hydrogel matrix, filled with either superparamagnetic or remanent magnetic nano-particles. Our microfluidic cartridges have a laminated structure consisting of a substrate with cilia, a PDMS interlayer defining a micro-channel structure, and a closing optical part (either glass or polycarbonate). These three layers are held together using a vacuum. The latter provides easy assembling and disassembling of the cartridge, enabling multiple reuse of the channel layer and the optical part. In order to have optical access in the vertical plane some cartridges were fabricated with a micro-prism having one reflective surface. It turned out that these optical structures are suitable for side-view µPIV measurements as well as inspection of the shape and movement of the cilia.

The fabrication of the magnetic actuator was made following two routes. One in which a permanent magnet is rotated with a given angular velocity and another one in which the magnetic field is generated by an electromagnet with four poles. The use of the electromagnet allows the modification of the field amplitude while being able to have the field uniform and under a controllable angle. The use of the permanent magnet however gives the possibility to obtain larger actuation fields at frequencies up to 60Hz. These facts will have to be taken into consideration when a magnetic cilia array is to be integrated in a lab on a chip cartridge.

To illustrate the possibilities of the system, we have presented an experiment in which the cilia are actuated in magnetic field with and without background flow.

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