Chapter 1
Introduction

1.1 Lab-on-a-chip

Lab-on-a-chip (LOC) is a technology that aims at performing analyses of biological samples (such as blood and urine), conventionally performed in a clinical lab, on a small chip. The analyses range from simple tests on biological samples to sophisticated DNA and cell analysis. The primary reason for the development of LOC is that the reduction in the sample size of the analyte enhances control and accuracy of bio-chemical reactions. The added advantage of the small size of the device is that smaller amounts of analyte and reagents are needed to perform the reactions, and the device becomes portable. As the facilities needed to perform the bio-chemical analysis are encapsulated in a small device, it was aptly named a micro-total analysis system (micro-TAS) by Manz *et al.* (1990).

The important tasks performed in a micro-TAS are the treatment of an analyte with suitable reagents, subsequent chemical reactions, separation of the molecules resulting from the reactions and the detection of these molecules. One example of bio-chemical reactions is a polymerase chain reaction (PCR), which is performed to amplify the concentration of specific DNA strands by orders of magnitude. In this process, the analyte (containing a few DNA strands) is subjected to cyclic heating and cooling. When a PCR is miniaturised, because of the small sample size, the thermal response time becomes low leading to a drastic reduction in the PCR cycle time (Kopp *et al.*, 1998). In another example, it was shown that the miniaturisation can lead to very rapid separation of bio-molecules using methods such as electrophoresis and chromatography (Manz *et al.*, 1994; Harrison *et al.*, 1993).

The micro-TAS can be encapsulated into a hand-held device which can be used for point-of-care (POC) testing, where the clinical diagnosis can be performed at the location of the patient even by an untrained person. Instruments to perform simple analyses such as to measure glucose levels (e.g. Bayer Contour, see www.bayercontourusb.us), hemoglobin levels (e.g. Hemocue, see www.hemocue.com) and lithium levels (e.g. Medimate Multireader, see www.medimate.com) have been commercialised. The importance of POC testing is especially applicable to patients with Type I diabetes, for which self monitoring of blood glucose is considered as an integral part of their treatment (Klonoff, 2007). It is also proven that POC devices can reduce the mortality rates in critical care units of hospitals (Rossi & Khan, 2004). Such instruments are also of immense help in disaster-affected areas, where it is difficult to perform regular clinical tests (Kost *et al.*, 2006).

As the surface to volume ratio is high in a LOC, physical phenomena associated with surfaces (e.g. surface tension and electrokinetics) gain importance. In addition, as the length scales involved are small (typically less than a millimetre), the viscous

forces in the fluid dominate over the inertial forces leading to laminar flow profiles in typical micro-TAS (Squires & Quake, 2005). While some of these are beneficial, the presence of others are detrimental. For instance, the fabrication of devices to perform individual operations on a micro-TAS cannot always be done by simply downscaling conventional methodologies. Mixing of an analyte with another fluid is difficult to achieve in a microfluidic device due to the laminar nature of flow at these length scales. Another challenge is the pumping of fluids through the microchannels and testing chambers on a lab-on-a-chip. In some applications, a local control of the flow is also necessary, which calls for a localised pumping system that can be embedded into a microchannel.

The fluid propulsion in microfluidic systems is performed using three different approaches: (i) mechanical methods – such as external syringe pumps, peristaltic pumps (Pilarski *et al.*, 2005; Liao *et al.*, 2005; Grover *et al.*, 2003; Svensson *et al.*, 2010; Lai & Folch, 2011; Gu *et al.*, 2004) and membrane pumps, (ii) using the electrokinetic properties of the fluids – such as in electro-osmotic pumps (Zeng *et al.*, 2002; Litster *et al.*, 2010) and magneto-hydrodynamic pumps (Lemoff & Lee, 2000; Homsy *et al.*, 2000; Jang & Lee, 2000; West *et al.*, 2002) – and (iii) acoustic methods (Langelier *et al.*, 2009; Nguyen & White, 1999; Yeo & Friend, 2009; Y. Bourquin & Cooper, 2010). These fluid propulsion mechanisms have been developed only in recent years. On the other hand, nature has been using remarkable fluid propulsion mechanisms at micron length scales for the locomotion and fluid transport, which are primarily based on mechanical actuators that beat back and forth. In this work, we use principles inspired by natural systems to design a fluid propulsion system that can operate inside microchannels and controlled by external force fields.

1.2 Micron-scale fluid manipulation in nature

Micron-scale fluid manipulation occurs in nature for two main reasons: locomotion and fluid transport. These are often (but not always[1]) performed using hair-like motile appendages knows as cilia and flagella (Murase, 1992; Cooper & Hausman, 1992). The cilia can beat in two different ways. Firstly, the cilia on the external surfaces of organisms such as opalina beat in an asymmetric manner with a distinct effective and recovery stroke (see Fig. 1.1 (a)-(c)). During the effective stroke the cilia are straight and push large amounts of fluid, whereas during the recovery stroke they stay closer to the cell surface and pull back only a small amount of fluid. The net fluid propelled is in the direction of the effective stroke (see Fig. 1.1(c)). The hydrodynamic interaction causes adjacent cilia to beat out-of-phase leading to a wave-like motion which is commonly referred to as metachronal waves (see Fig. 1.1 (b)). Secondly, there is another category of cilia, called nodal cilia that are present on node cells of embryos and revolve with a whirling motion about an axis that is non-orthogonal to the surface on which they are attached (see Fig. 1.1 (d)-(e)). These cilia create a large flow when they are away from the surface and a low flow in the opposite direction when they are close to the surface. As a result, a net flow is created in the direction of the upward stroke. A flagellum that is attached to a cell propagates waves of transverse displacement along its length to exert a force on the fluid in the direction of the wave, which causes the flagellum and cell to move in the opposite direction (see Fig. 1.1 (f)-(g)).

[1] The locomotion of a Cyanobacterium takes place by propagating waves of lateral displacement along its surface (Ehlers *et al.*, 1996).

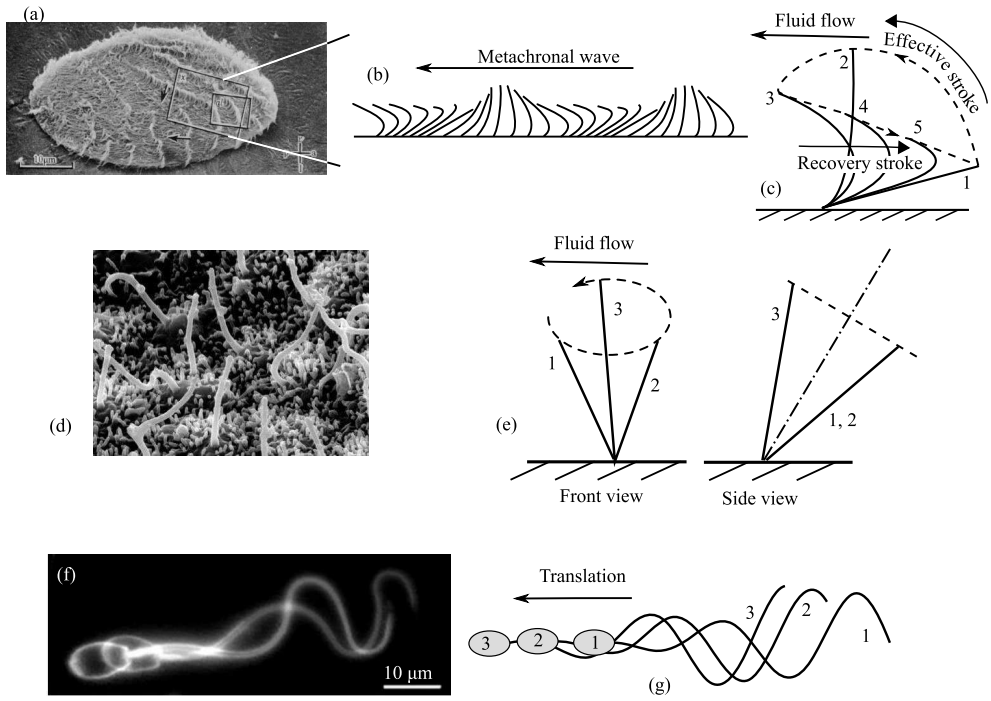

Figure 1.1: *(a) Micrograph of cilia on Opalina (Tamm & Horridge, 1970). (b) Metachronal waves are formed due to out-of-phase beating of the cilia. (c) Schematic diagram showing the movement of cilia. (d) Micrograph of cilia on node cells (http://www.physics.ubc.ca/steve/research/A-TopProt.html). (e) Schematic representation of the motion of nodal cilia. (f) and (g) Snapshots of a flagellum attached to a sperm cell (Woolley, 2010). The numbers refer to the sequences in time. The flagellum creates a wave of transverse displacement for fluid propulsion, whereas the cilia in the case of Opalina beat with a distinct effective and recovery stroke, and in the case of node cells move such that they describes a cone.*

Flagella are primarily known for locomotion (Lighthill, 1976; Brennen & Winet, 1977), whereas the cilia perform different functions like locomotion and fluid transport (Murase, 1992; Brennen & Winet, 1977; Gardiner, 2005). In some cases, the cilia are non-motile and perform sensing function (Gardiner, 2005; Malone *et al.*, 2007). The flagella are usually attached to a cell body, whereas the cilia occur in groups over the surface of microorganisms (for example on the surface of a Paramecium) or tissues of organs. The cilia are associated with micro- as well as macroorganisms. Microorganisms (such as Paramecia, Opalina and Centophores) have cilia on their outer surface for locomotion. In organisms such as Lophophorates, the cilia create a water current that brings suspended food particles near the organism's mouth (Strathmann, 1973). The nodal cilia create a fluid flow that initiates the left-right asymmetry during embryonic development (Halbert *et al.*, 1976; Ibanez-Tallon *et al.*, 2003). The cilia on the surfaces of ventricular system of the brain play an important role in the transport of the cerebro-spinal fluid (Roth *et al.*, 1985; Ibanez-Tallon *et al.*, 2003). Cilia are also present in the inner-lining of the respiratory tract and propel mucus out of the lungs.

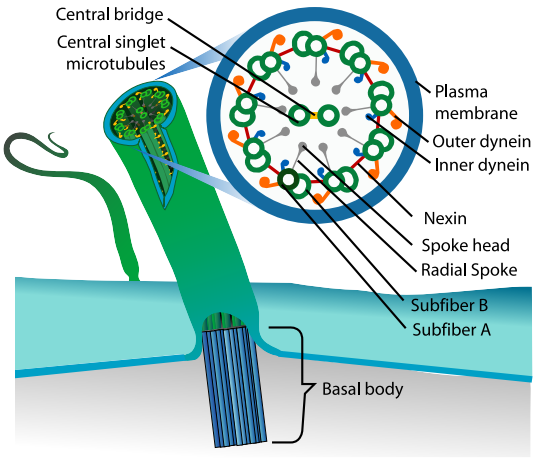

Figure 1.2: *Cartoon showing the microstructure of the axoneme (www.en.wikipedia.org/wiki/cilium).*

1.3 Structure of cilia and flagella

The cilia and flagella are appendages that protrude from the cell surface (see Fig. 1.2). They have a similar microstructure, the axoneme, which consists of a circular array of nine microtubular doublets (composed of microtubules A and B) that are connected together with the linker proteins called nexin. At the centre of the doublet array two microtubules are connected through rigid links to form the central pair. Each doublet is connected to the central pair through radial spokes. The axoneme is driven by the ATP-powered motor protein dynein that are attached to the inner and outer sides of microtubule A. During an operation cycle, the dyneins attach themselves to microtubule B of the adjacent doublet and exert a force such that two adjacent microtubular doublets slide relative to each other (Sale & Satir, 1977). As the axoneme is fixed to the cell at its base, this sliding is translated into bending of the axoneme. Interestingly, local actuation of the dyneins creates a global beating of a flagellum or a cilium. However, a clear picture of how the dynein actuation is translated into a global motion of the cilia and flagella is not yet available. The typical length scales associated with cilia and flagella are as follows. The length of cilia is usually between 10 and 20 microns, whereas flagella can have a length up to 200 microns. The diameter of the axoneme is 250 nm. The distance between two inner and outer dyneins along the length of a microtubule is 96 nm and 24 nm (Ibanez-Tallon *et al.*, 2003), respectively. In the non-motile and nodal cilia the central pair is absent, while in the former also the motor proteins are absent.

1.4 Hydrodynamics at small length scales

At the small length scales of cilia and flagella (up to hundreds of microns), viscous forces dominate over the inertial forces leading to low values in Reynolds number[2] (Re $<<$ 1)- the ratio of fluid inertial forces to the viscous forces. To get a feel for the viscous forces, consider a microorganism swimming at a steady velocity. The distance (normalised to

[2]For cilia of length 10 μm that beat at a frequency of 20 Hz the Reynolds number is 2×10^{-3}.

its body length) this organism continues to move after its propulsion mechanism has stopped scales with Re^3 (Lauga & Powers, 2009). Since $Re << 1$, the organism cannot coast after it has stopped its propulsion mechanism. This is because the viscous forces are so huge that they dissipate the kinetic energy of the organism instantaneously.

There are two consequences of high viscous forces. Firstly, a certain class of cyclic actuator motion – called reciprocal motion, in which the forward motion is exactly the same as the backward motion – cannot lead to a net fluid propulsion. An example of reciprocal motion is the back and forth oscillation of a rigid rod about one of its ends. During a reciprocal motion of the actuator the flow created during the forward motion of the actuator will be cancelled by the flow of the backward motion, creating zero net fluid transport. Secondly, the fluid behaviour is rate independent, i.e., even if the forward reciprocal motion of the actuator takes place faster than its backward motion, there will be no net fluid flow. The flow created by the fast forward motion will be exactly cancelled by the slow reverse motion of the actuator. Rigorous mathematical proofs of these two properties can be found elsewhere (Childress, 1981; Lauga & Powers, 2009).

As the cilia and flagella are able to create a fluid flow, their motion should be 'non-reciprocal'. The cilia motion is non-reciprocal because of its distinct effective and recovery stroke. In the case of flagella, the wave of transverse displacements causes the slope of any segment before and after it has reached a maximum displacement to differ by a sign. This makes the flagella motion to be non-reciprocal. The non-reciprocal motion can also occur at larger length scales. For instance, a Cyanobacterium swims through a fluid by propagating waves of lateral displacement on its surface. Individual points on the surface oscillate about a mean position in a reciprocal fashion, but the directional wave of lateral displacements makes the surface motion non-reciprocal (Stone & Samuel, 1996).

1.5 Objective of the thesis

The goal of this thesis is to mimic the non-reciprocal motion of natural cilia in order to propel fluids through microchannels. As the local dynein actuation mechanism is not yet fully understood, we take an alternative approach. The idea is to design artificial cilia that are attached to the inner surface of microchannels and to actuate them with externally-applied force fields (not through dynein-like internal forcing), so that they can beat in an asymmetric motion and create a fluid transport. These artificial cilia can be realised using thin polymer films with embedded magnetic nano-particles that can respond to an external magnetic field, see Fig. 1.3. Depending on the nature of the magnetic particles the film can be either paramagnetic or permanently magnetic.

The objective of this thesis is two-fold: Firstly, to identify for what geometries, material properties and applied magnetic fields a magnetic film will mimic the asymmetric motion of natural cilia. Secondly, to identify how the created flow can be enhanced by controlling different factors such as the cilia geometry, magnetic field, cilia spacing, channel height, fluid inertia and out-of-phase motion. We answer these questions using numerical models that capture the physical behaviour of magnetically-driven artificial cilia. These numerical models predict the cilia deformation and the resulting fluid flow by solving the Maxwell's equations, solid dynamics equations and Navier-Stokes equations in a fully-coupled manner.

[3] Assuming the mass density of the organism is the same as the fluid.

1.6 Thesis outline

The outline of the thesis is as follows. In chapter 2, we derive the equations of a two-dimensional coupled solid-fluid magneto-mechanical model used to simulate the cilia motion. We model the cilia as elastic Euler-Bernoulli beams taking into consideration geometric non-linearity and inertia of the cilia in a Lagrangian framework. The magnetic field is calculated by solving the Maxwell's equations using a boundary element approach. The Navier-Stokes equations, which capture the behaviour of the fluid flow, are solved within an Eulerian setting for the velocity and pressure using finite elements. The solid-fluid coupling is performed by imposing the no-slip condition at the nodal points of the Euler-Bernoulli beam elements using Lagrange multipliers within a fictitious domain framework. The physical dimensionless parameters that govern the behaviour of the artificial cilia are derived from the principle of virtual work.

A number of artificial cilia configurations that show an asymmetric beat motion are designed by choosing different material properties, initial geometries and magnetic fields in chapter 3. The fluid flow created by these cilia is analysed in the limit of low Reynolds number (Stokes regime). The performance of the cilia is also analysed in terms of dimensionless parameters, introduced in chapter 2, which leads to the parameter space in which the cilia can perform optimally.

The flow and pressure generated are typical parameters that specify the characteristics of any pumping device. These are analysed as a function of the cilia spacing and channel geometry in chapter 4. It will be shown that the flow and pressure generated increase when the cilia spacing is decreased. However, when the channel height is decreased, the flow generated decreases, while the pressure increases. This chapter provides guidelines for selecting the optimal channel size, cilia spacing and cilia length for a specific channel morphology.

Literature suggests that the performance of cilia can be improved for mixing applications by exploiting inertial forces. In chapter 5, we analyse the effect of inertial forces on the fluid transported by the artificial cilia. It will be shown that the presence of inertia brings in many interesting phenomena. The flow created by the cilia can be significantly larger than in the Stokes regime, and it becomes unidirectional in some cases. The flow created is due to the combination of the asymmetric area and temporal asymmetry (char-

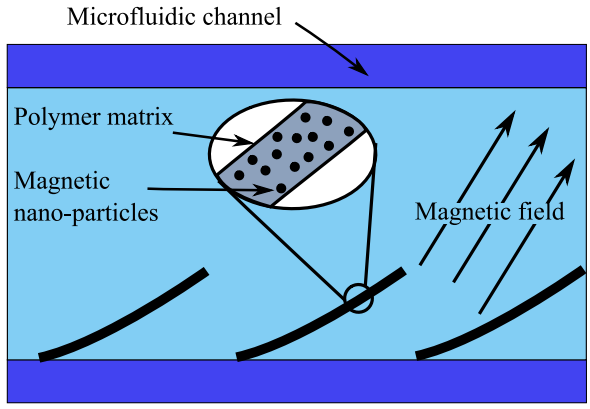

Figure 1.3: *Schematic picture of the approach taken. The artificial cilia consist of a polymer matrix with embedded magnetic nano-particles. The cilia exhibit an asymmetric motion when a tuned external magnetic field is applied.*

acterised by a slow effective and fast recovery stroke). In order to delineate the effect of asymmetric area, temporal and orientational (characterised by the asymmetry of the cilia motion with respect to the microchannel) asymmetries we study the flow created using a model problem in which the individual contributions of these three asymmetries can be identified (chapter 6).

In nature, adjacent cilia beat with a phase difference (see Fig. 1.1 (b)), which will induce an additional non-reciprocating motion, on top of the asymmetric motion of individual cilia. This leads to the formation of metachronal waves. In chapter 7, we analyse the effect of magnetically-induced metachronal waves on the fluid transport created by the cilia.

In chapter 8, we explore the principle used by Cyanobacteria for fluid transport: collective non-reciprocal motion. The cilia are made to oscillate about a mean position in a reciprocal manner, but such that they have a phase difference with their neighbours. This causes the cilia motion to be collectively non-reciprocal. We investigate the physical mechanisms that cause the fluid transport and find under what conditions the flow created reaches a maximum value.

So far we used a two-dimensional numerical model to simulate the fluid transport caused by the cilia. This framework assumes that the width of the cilia is much larger than the cilia length and channel height. To explore the effect of cilia width and out-of-plane motion on the resulting flow, a three-dimensional numerical model is developed in chapter 9. The flow created by an array of cilia with the metachronal waves travelling in and perpendicular to the beating plane is also investigated.

The main results of the thesis are finally summarised and guidelines for the optimal design of the artificial cilia are given.