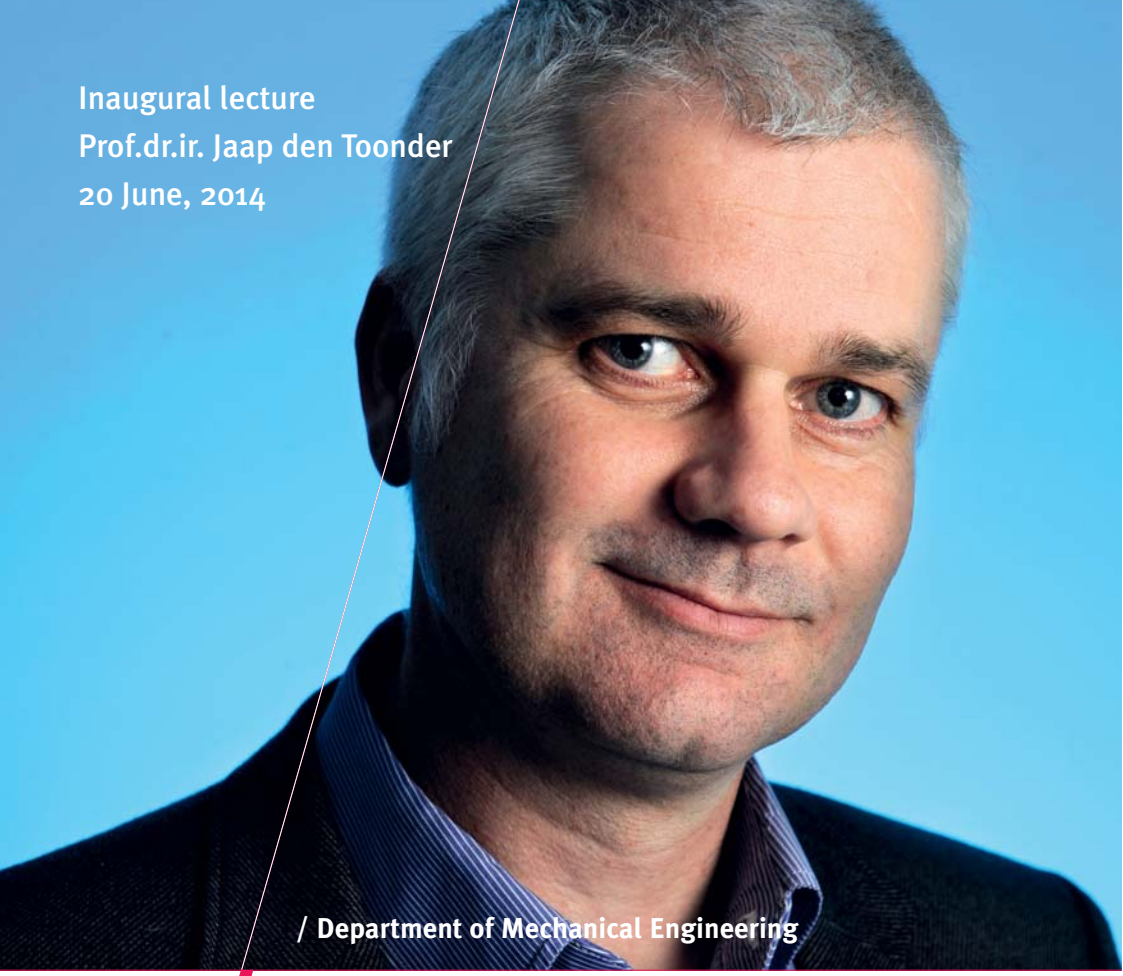
Microfluidics, merging technology and biology



Inaugural lecture
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20 June, 2014

/ Department of Mechanical Engineering

TU/e Technische Universiteit **Eindhoven** University of Technology

Microfluidics, merging technology and biology

Where innovation starts

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Presented on 20 June, 2014
at Eindhoven University of Technology

Introduction

An important recent development within the field of Microsystems has been the emergence of ‘wet’ microsystems: microfluidic devices with tiny channels and chambers in which fluids can be precisely manipulated and analyzed. Originally based on technologies from the semiconductor industry, microfabrication methods for microfluidics have undergone a transition to approaches that do not require an expensive cleanroom and that use unconventional materials like elastomers and thermoplastics.

In this lecture, I will show how this development stimulates the exciting merging of engineering sciences and life sciences. On the one hand, fluid manipulation principles found in biology can be adopted and applied in microfluidic devices through ‘biomimetic design’. On the other hand, microfluidic devices can be used to control and understand biological processes by studying biomolecules, biological cells, or even miniature models of complex tissue mimicking true-to-nature human organ function. Eventually, this will expand our understanding of health and disease and impact medical diagnosis and therapy.

Microfluidics

Microfluidics is the science and technology of manipulating, processing and analyzing small volumes of fluids. “Small” means that the typical dimensions of the fluid volume are about a millimeter down to micrometers. Think of a raindrop, which usually has a diameter of about a millimeter. A cubed millimeter of volume equals one microliter.

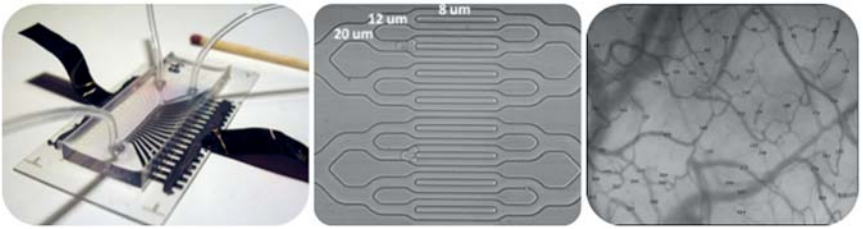

Figure 1

Microfluidic systems. Left: a microfluidic device with a Y-shaped channel with a width of 1 mm, containing electrodes to generate electrical fields. Middle: zoomed in on a microfluidic device showing a micro-channel network with channel widths down to 8 micrometers. Right: vascular network of blood vessels.

To be able to manipulate and analyze such small fluid volumes, microfluidic devices are used that contain tiny channels and chambers, as illustrated in figure 1. The leftmost image shows a device with a Y-shaped channel with a width of a millimeter. Tubes are connected to the device for the fluid to enter and leave, and the device contains electrodes for generating electrical fields in the channel to manipulate the fluid. The middle picture is a blow-up of a microfluidic channel structure, where the smallest channels have a width of less than 10 micrometers. The right panel in figure 1 is an image of a biological microfluidic system that is present in our own bodies, namely the vascular network formed by our blood vessels. This system consists of tubes ranging in size between micrometers and millimeters, supplying nutrients, gases and hormones to all organs throughout our body, and removing waste and byproducts when needed. Billions of red blood cells, white blood cells and platelets circulate continuously through this vast microfluidic system, each having their own specific role.

The resemblance in typical size between the channels in man-made microfluidic devices and the blood vessel in the vascular system is not accidental: it means that microfluidic devices are ideally suited to control and study biological processes by studying biomolecules, biological cells, or even miniature models of complex tissue. The dimensions are compatible – cells have typical sizes between approximately 10 and 20 micrometers, depending on cell type. Later, we will see some concrete examples of this important application of microfluidics.

It is crucial to be able to control the fluid flow in microfluidic devices. Fluid flow at these small scales is special, and it is different from what we are used to from our daily experience at larger scales. One important property of microfluidic flows is that they are almost always laminar, as opposed to large-scale flows that are most often turbulent. Turbulent flow is characterized by a chaotic whirling motion over a cascade of length scales. This can be nicely observed when we mix milk in our coffee by stirring, in which the turbulent flow provides a very effective way of mixing (see figure 2). At small length scales, however, viscous effects immediately suppress small flow disturbances, and therefore turbulence does not get the opportunity to develop, and the fluid flow is always nicely behaved and predictable. Stirring at the microscopic scale is like stirring in highly viscous syrup. As illustrated in figure 2, when two fluid streams come together in a microchannel they flow alongside each other without any unpredictable turbulent flow, and no mixing occurs.

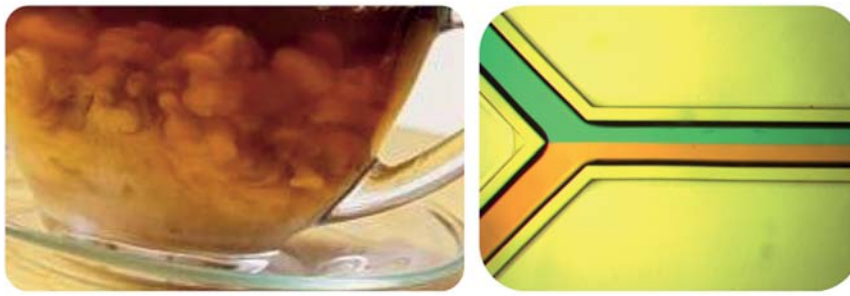

Figure 2

Left: to mix milk in our coffee we create turbulent flow in the cup. Right: in contrast, in microfluidic systems, fluid flow is almost always laminar since disturbances are immediately suppressed at small scales, and fluids stream flow alongside each other.

This property of laminar flow at small scales is both an advantage and a disadvantage. On the downside, when mixing is needed such as in fast chemical reactions, special tricks must be applied in microsystems to create chaotic flow to

enhance the mixing. On the positive side, the fact that microfluidic flow is always well behaved and predictable means that it can be controlled, and well defined flow patterns and gradients can be created. This possibility of ultimate control has opened up important applications of microfluidic devices, as we will see later.

In addition to the laminar nature of microfluidic flow, there are other differences with large scale fluid flow: physical effects that are negligible at large scales become important or even dominant at small scales, and can be used for flow control. Surface tension, the force that keeps a water droplet together, is very strong at sub-millimeter scales. Therefore many microfluidic devices are based on manipulation of droplets, or make use of capillary effects to pump fluids. Also, electrical fields created in the devices, for example using integrated electrodes, can be used to pump fluids (by electro-osmosis), to transport charged particles (by electrophoresis) or even move biological cells (by dielectrophoresis). Similarly, acoustic waves generated in the microchannels can be used to create flow patterns or move particles or cells. Magnetic fields are often used to control the motion of magnetic microparticles in microfluidic devices, and even light can be used for microfluidic control. This means that there is a whole toolbox of methods that can be used to manipulate fluid flow in micro-devices.

The development of the field of microfluidics started in the early 1990's, with initial applications in chemical analysis. It enabled the introduction of micro-scale analytical methods – gas-phase and liquid chromatography for the separation of mixtures and capillary electrophoresis for the separation of molecules – which, in microfluidic format, revolutionized chemical analysis by making it possible to simultaneously achieve high sensitivity and high resolution using very small amounts of sample.[1] In the past decade, the number of applications in which microfluidics plays a role has been growing: in medical diagnosis for example, where microfluidics enables miniaturization and integration of diagnostics processes for detecting proteins or nucleic acids in a very small amount of complex fluids like blood. This could ultimately result in the 'lab-on-a-chip'. Another example is its use as a research tool to study biological systems and processes, and develop understanding about health and disease.

In the remainder of this lecture, I will talk about the technology developments that have boosted the field of microfluidics, and that have in particular stimulated its application to answer biological and medical questions. On the other hand, we will see how lessons from biology can be used to develop the technology itself.

Technology and materials

The technologies used originally to realize microfluidic devices were first developed for the semiconductor industry and later expanded by the micro-electromechanical systems (MEMS) field. Hence, early microfluidic devices were fabricated from silicon and glass using cleanroom techniques that were translated to microfluidic device fabrication. This was largely a choice of convenience (because the techniques and facilities were already in place) and necessity (since early microfluidics focused mainly on electrophoretic phenomena where glass is a preferred material). In the 1990's, it was believed that these cleanroom techniques would boost the development of microfluidic chips, just as they had done for electronic integrated circuits chips (IC chips). However, at some point in time it became clear that the broad development of microfluidic technology and its application to biology were in fact hampered by the nature of these techniques: on one hand, cleanroom facilities are very expensive and inaccessible to many researchers and, on the other hand, for analyses of biological samples in water, devices fabricated in glass and silicon are usually unnecessary and inappropriate. Moreover, for most applications in biology, there is simply no need to have the sub-micrometer resolution of cleanroom fabrication.[2]

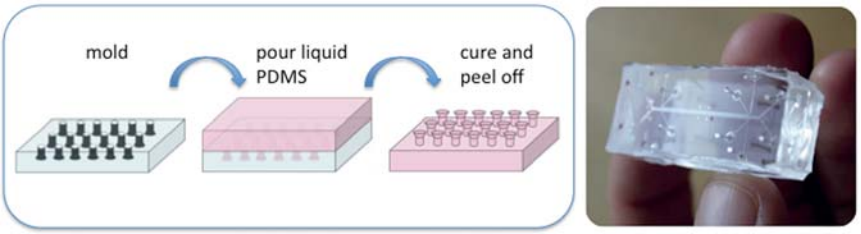

Figure 3

Left, the basic steps of soft lithography. Starting with a mold, liquid PDMS is poured on the mold replicating the mold structures, the PDMS is cured at high temperature and released from the mold by peeling off. Right: a microfluidic device made completely of PDMS (courtesy Rob Tack).

The introduction of 'soft lithography' into the field of microfluidics, by the end of the 1990's, changed the scene and has had an enormous impact, contributing to the growth of the field in both technological development and number of publications.[3] Soft lithography is based on processing the material polydimethylsiloxane (PDMS), or simply silicon rubber, which is a soft, optically transparent, gas- and vapor-permeable elastomer. PDMS can be cast on a mold into the required shape in liquid form, solidified using a temperature treatment, released from the mold by peeling off, and then bonded to another substrate to form the final microfluidic device, see figure 3. This process can be done with a relatively cheap and easy set-up, making it possible to fabricate small numbers of devices in a university setting rather than in an advanced cleanroom. An important element is obviously the mold, which contains the negative of the microfluidic structure to be replicated by the PDMS. For very small feature sizes, the mold must still be made, for example in silicon or glass, in a cleanroom environment. However, for feature sizes of micrometers or larger, approaches using basic photolithography, laser micro-fabrication or even mechanical micro-milling can be used to produce the mold in glass or a polymer material, making the whole procedure a truly 'out-of-cleanroom' fabrication process – see figure 4.[4] Apart from ease of processing, several other key factors were important for the adaptation of PDMS in microfluidics technology: its optical transparency offering the ability to observe processes directly within the device; its flexibility, making it possible to create valves and actuators, using pneumatic actuation; and its compatibility with cell culture.

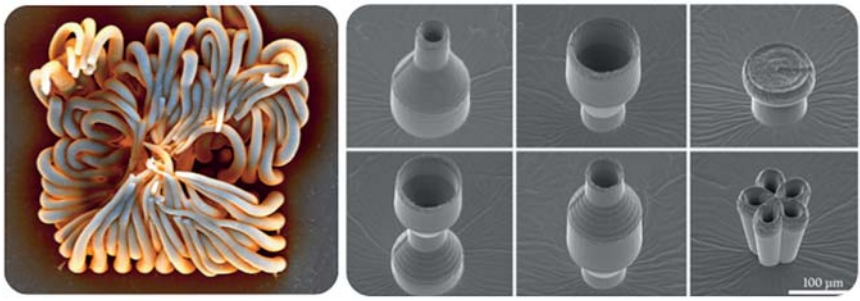

Figure 4

Examples of micro-molded PDMS structures made in our out-of-cleanroom lab. The mold was made by creating the negative of these (3-D) structures in glass using a femtosecond laser. The left picture shows a bundle of tiny PDMS hairs containing magnetic nanoparticles (length about 100 micrometers, diameter about 10 micrometers); courtesy Ye Wang. The right picture shows truly three-dimensional structures of PDMS tens of micrometers in dimension.[4]

PDMS also has disadvantages, such as the permeability to gases and liquids for some applications or the fact that PDMS easily absorbs some small molecules. Also, although the ease of PDMS processing makes it very suitable for testing new microfluidic concepts quickly, the large-volume manufacture of PDMS devices is not straightforward since high-throughput methods such as injection molding cannot be easily used for PDMS.

The limitations of PDMS have stimulated researchers and product developers to explore alternative materials in recent years. Thermoplastic polymers such as polymethylmetacrylate (PMMA, or Plexiglas), polystyrene (PS) and polycarbonate (PC, the material from which CD's are made) were already being used by some R&D groups in the 1990's but have been gaining more general attention from the microfluidic community lately. These materials, while still having excellent optical transparency, are much less permeable than PDMS, and they also exhibit much less absorption of small molecules. And, very important for any industrial-scale applications, thermoplastic devices can be mass-produced using established injection molding methods. Interestingly, companies like Sony DADC, with large-scale injection molding facilities that were originally set up for the production of CD's and DVD's, are now entering the market of microfluidic device manufacturing, looking for new applications of the technology following a decline in the optical storage business with the advent of Internet.[5] There are even examples of injection molded microfluidic devices that are in an advanced stage of product development, such as the handheld 'Minicare' diagnostics system developed at Philips.[6] On a research lab scale, injection molding is quite expensive but more accessible fabrication techniques like hot embossing or laser manufacturing could be used for thermoplastic polymers in such a setting. One of the disadvantages of thermoplastic polymers is that they are much less flexible than PDMS and therefore it is more difficult to realize valves and actuators.

Another recent development that should not go unmentioned is that of 'paper microfluidics'. In fact, this expands on tried–and-tested approaches that are already applied commercially, as in pregnancy testers. The basic idea is that fluid flow is created since a fluid naturally wicks through the fibrous structure of paper due to capillary forces, at least if the paper is made hydrophilic. Patterning of hydrophilic and hydrophobic regions on the paper can create 'channels' that guide the transport. The hydrophobic channel patterning can be accomplished using a variety of methods, such as wax printing, photolithographic patterning of photoresists, and flexographically printed polystyrene. A main motivation that is often given in favor of paper microfluidics is the low cost of the paper with respect

to other materials. This argument is questionable since, in the end, for a real microfluidic product the main cost is usually not in the materials for the device, but in the manufacturing process, the reagents and the packaging for example. Also, the nature of the material will limit the sensitivity of medical test to be carried out with a paper device.

From this story, it is clear that microfluidic technology has evolved from semiconductor technology (based on photolithography on glass and silicon) to a number of different technologies including soft lithography using PDMS, thermoplastic polymer processing and paper technology. All of these have advantages and disadvantages. When electric functionality is needed, glass or silicon is the most suitable base material whereas when mechanical actuation is required, PDMS is the current material of choice. Soft lithography is ideally suited for fast prototyping a limited number of devices while for high-volume production, injection molding of thermoplastic materials can be used. Most probably, in the future microfluidic devices will become increasingly 'hybrid', that is they will not be made with a single technology but with combinations of different technologies so that different functionalities can be integrated. However, especially in the research and development phase of a microfluidic device, and particularly in a university setting where novel concepts need to be experimentally tested, the approach will be 'out-of-cleanroom', since only then can accessibility, speed of the manufacturing cycle and flexibility of material choice be guaranteed.

The introduction of a new class of materials known as 'responsive' or 'smart' materials in microfluidics technology opens new and exciting possibilities for active control of microfluidic flow. Such materials respond to an external stimulus by changing shape or volume. The applied stimulus may be illumination by light, a change in temperature, a magnetic or an electrical field, or a change in pH. A classic responsive material is a piezoelectric material like lead zirconate titanate (PZT), which expands upon applying an electrical field. This material is used in acoustic transducers and inkjet print heads but it is difficult to integrate the material in microfluidic devices. New classes of responsive materials are being developed based on polymers, and these can be processed using non-cleanroom techniques like micro-molding, printing or soft lithography, and are therefore much more suitable for microfluidic integration.

One example of such a new material is a magnetic rubber: PDMS with dispersed magnetic nanoparticles. The material responds to an externally applied magnetic field by changing shape, and can be processed with the usual tools available for

PDMS based fabrication. A second example is a liquid crystal network.[7] This material consists of liquid crystal molecules, cross-linked in a polymer network, see figure 5. The design of the structure of the liquid crystal molecules and the processing conditions allows the molecules to arrange themselves in an ordered fashion that is fixed by the surrounding polymer network. By applying an external stimulus, which can be illumination by light, a rise in temperature or a change in pH, the molecular order is disturbed. This results, on a larger scale, in a deformation of the material. A third example is a responsive hydrogel.[8] This is a polymer network that can contain a lot of water, see figure 5. The equilibrium state of the polymer network can be disturbed by an external stimulus whereby the material takes up or expels water, which results in extensive swelling or shrinking of the material. Together with groups in Chemical Engineering, we are working on all of these materials.

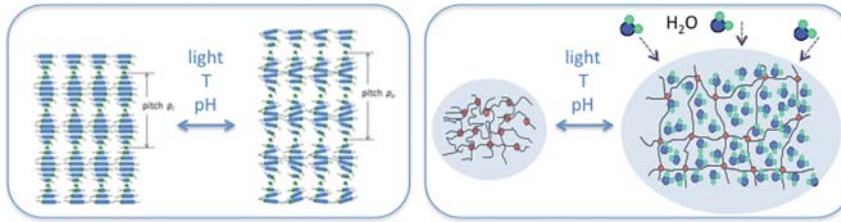

Figure 5

Two classes of responsive materials. Left: a liquid crystal network, that changes shape due to a loss of molecular order induced by an external stimulus (light, temperature, pH). Right: a hydrogel that swells or shrinks upon applying a stimulus, talking up or repelling water from its network. Courtesy Danqing Liu.

The ability to integrate these responsive materials in microfluidics design and fabrication creates many exciting possibilities both for control of fluid flow and for applying general mechanical actuation in microfluidic devices which, as we will see later, is essential for studying biological phenomena. In the next section, I will discuss some applications of responsive materials in microfluidic systems in more detail.

Biomimetics: active microfluidic control

Controlling fluid flow in microfluidic devices is key. As mentioned earlier, to achieve this control, the small-scale nature of microfluidics offers many possibilities that cannot be applied to macroscopic flows. Vice versa, some effects at the macroscopic scale cannot be applied at small scales. Inertia is perhaps the most important effect that is almost absent at small scales. Inertia is the property of an object to maintain a constant velocity, unless an outside force acts on it. An object with a large inertia will strongly resist a change in velocity or, in other words, it is difficult to start or stop its movement. An object with little inertia, on the other hand, will almost instantaneously start or stop when acted upon by some external or internally generated force. Inertia is actually the effect that also causes turbulence in fluid flow, which is almost always absent at microfluidic scales as we have seen before.

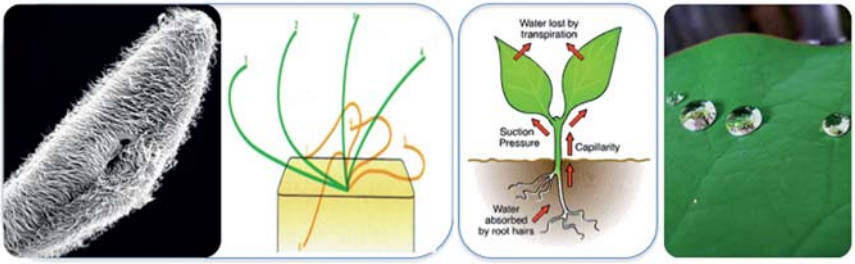

Figure 6

Microfluidic control in nature. Left: microorganisms like paramecium swim by oscillating micro-hairs called cilia by which their surfaces are covered. Middle: plants transport water by a combination of osmotic pressure, capillary action and evaporation. Right: droplet contact angles are controlled by surface topography on Lotus leaves.

Creative solutions to technical problems can often be inspired by how nature has solved similar problems – this approach is called “biomimetics”: mimicking biology. Nature has devised many different ways of creating fluid flow, many of them for animal propulsion, that is, for flying or swimming. At larger scales, examples are the flapping wings of birds, and the waving tails of fishes – however these cannot be translated to a microfluidic setting since they make use of inertia.

At really small scales, however, typically for sub-mm sizes, nature has also devised a whole range of different approaches to fluid manipulation (see figure 6 for some examples).

One small-scale fluid manipulation mechanism used by nature is that by cilia or flagella. Cilia can be viewed as small hairs or flexible rods, with a typical length of between 2 and 15 micrometers. They cover the outer surface of microorganisms such as paramecia, shown in figure 6. The cilia move back and forth in a concerted manner, and are very effective in generating flow: the swimming speed of paramecium, for example, can be approximately 1 mm/s (i.e. it can travel a distance of 10 times its own body length in a second). As shown in figure 6, the motion of an individual cilium is asymmetric, which is needed because of the absence of inertia: if its movement were perfectly symmetric, it would just displace fluid back and forth to the same position, even if it moved fast in one direction and slowly back. Cilia can be found abundantly in nature, also in our own bodies: in the lining of human lungs and the windpipe (trachea) to sweep mucus and dirt out of the airways in order to avoid infections, and also the Fallopian tube of females is covered with cilia that move the fertilized ovum from the ovary to the uterus, where the ovum attaches itself.[9]

Another inspiration for microfluidic fluid control is how plants and trees transport water, taken from the soil, up to their leaves (see figure 6). In fact this happens through the combination of three different effects; (1) The osmotic pressure at the roots drawing the water in from the soil; (2) capillary action driving the water up capillaries ('xylem') within the stem, which is enhanced by branching of the capillaries; (3) evaporation through small openings ('stomata') on the leaf surface.

Finally, I would like to mention here the variety of surface topographies that can be found in nature and that control fluid flow near the surface. A well-known example is the Lotus leaf whose very specific microscopic surface structure makes it super-hydrophobic (see figure 6). This means that water droplets sitting on this surface have a perfectly spherical shape, and very easily roll off. In doing this, the droplets take up and transport dirt from the leaf, which makes this surface ' self-cleaning'. Another example of surface topography is the skin of sharks, which contains a particular structure that controls the fluid flow boundary layer near the skin such that the hydrodynamic drag is reduced.

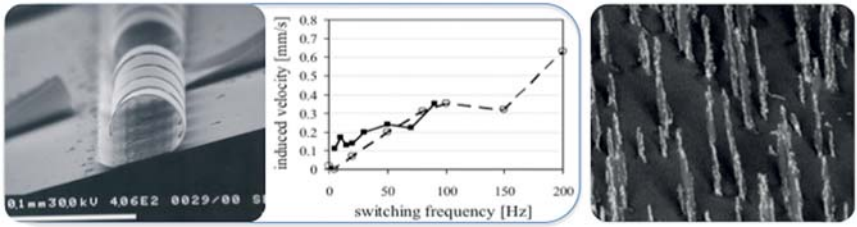

Figure 7

Artificial cilia. Left: electrostatic artificial cilia (length: 100 micrometers) that, when integrated in a microfluidic device (figure 1, left), generate substantial fluid flow velocities.[11] Right: magnetic PDMS artificial cilia fabricated using a non-cleanroom technology; their length is about 100 micrometers, and when actuated in water they generate flow speeds of approximately 100 micrometers per second.[14]

All these biological principles have inspired scientists to develop microfluidic flow manipulation technologies. One example is the recent development of artificial cilia that have been applied to pump and mix microfluidic flows.[10] Such artificial cilia are not literal copies of the biological cilia, but they resemble the appearance and principle. Figure 7 shows some examples of artificial cilia developed in our lab. The electrostatic cilia are curled polymer flaps covered with an ultrathin metal film that are about 100 micrometers long and can be actuated by applying a voltage: the cilia roll out over the surface when applying a voltage and they spring back elastically when the voltage is turned off. An oscillatory motion results when the voltage is switched on and off repeatedly. When integrated in a microfluidic device (figure 1, left), substantial fluid flow velocities are generated (up to 0.5 mm/s) and by using certain configurations chaotic fluid mixing is achieved.[11] Similar artificial cilia were made out of a rubber material with dispersed superparamagnetic nanoparticles that could be actuated using a rotating magnetic field.[12] The disadvantage of these approaches was that advanced and expensive cleanroom technologies were used to make them.

Therefore, recently, we started to develop non-cleanroom approaches to making magnetic artificial cilia, on the basis of self-assembly of magnetic micro-particles,[13] using soft lithography (figure 4, left panel) magnetic fiber drawing,[14] and a roll-to-roll process. Figure 7, right panel, shows artificial cilia made by magnetic fiber drawing of magnetic rubber (PDMS). Integrated in microfluidic devices, these artificial cilia can generate flow, create chaotic flow patterns for microfluidic mixing and also transport species such as biological cells.

Capillary and evaporative microfluidic flow control, inspired by plants and trees, has also been applied in many microfluidic devices. The attractiveness of these mechanisms is that they do not require external energy, that is, they are autonomous and run by themselves. Microfluidic channels can be designed for capillary pumping by appropriately tuning surface energy and geometry. Applying a porous structure at the exit of a microfluidic channel network, mimicking the stomata, can create an evaporative pump. The fluid evaporates through the pores, which drives the flow in the microfluidic channels.[15]

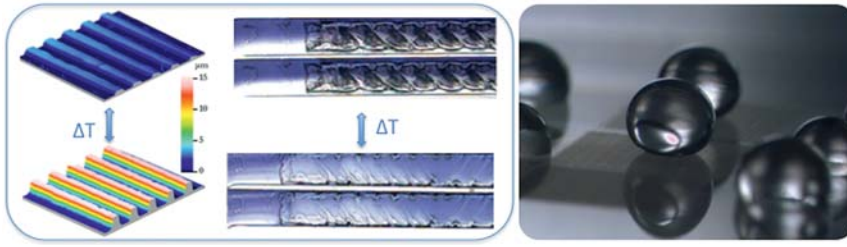

Figure 8

Left: a patterned hydrogel is used to create a surface topography that can be switched by temperature; integrated in a microfluidic channel, mixing can be controlled (courtesy Hossein Amirabadi). Right: controlling surface topography by laser treatment of PDMS determines the contact angle of droplets.[16]

Finally, dynamic surface topographies can be made based on responsive materials, liquid crystal networks or hydrogels. These surfaces can be patterned using lithography, creating a surface topography that can be switched on or off repeatedly by an external stimulus. Depending on the chemical composition, we can use light, heat or pH as a trigger. Figure 8 shows a hydrogel surface that can be switched, using temperature, between an almost flat state and a topography consisting of ridges with a height of 15 micrometers. This material was integrated into a microfluidic channel to switch slanted ridges on the channel floor on and off. Using this approach, it was possible to switch microfluidic mixing within the channel on and off, as shown in Figure 8. Inspired by the Lotus leaf, superhydrophobic surface patterns have been made by a straightforward process of laser treating a PDMS surface (figure 8).[16] In combination with responsive materials, dynamic surfaces can be created on which liquid droplets can be actively manipulated.

Biological applications

As we have seen earlier, initial applications of microfluidics were in the domain of chemical analyses. Over the years, other application fields have been added. A main driver for the field has been the development in medical diagnostics. Conventionally, for medical diagnosis a patient sample (often blood) is taken and subsequently analyzed in a medical diagnostic laboratory, as sketched in figure 9. Depending on the nature of the analysis (detection of proteins, nucleic acids or other species), many operations must be carried out for the diagnosis to be concluded. The whole procedure consists of many sub-processes, carried out in the laboratory by people using instruments on lab benches and with fairly large amounts of sample and chemicals. Moreover, the whole process takes a relatively long time. Microfluidic technology enables a whole diagnostic test to be integrated into a hand-held device, and eventually the complete diagnostic laboratory integrated on a single chip the size of a credit card (see figure 9). All of the processes usually done in the laboratory now run in micro-reaction chambers that are connected by micro-channels. The sample volume may be as small as a few drops of blood. The advantages are that the diagnostic process is faster, cheaper, uses small amounts of sample and reagents, and can be carried out outside of the diagnostic lab, at the point of need or in low-resource settings such as developing countries.

Figure 9

Microfluidics enables the miniaturization and integration of medical diagnostics, towards a handheld immunosensor (courtesy Philips), and ultimately a lab-on-a-chip.

Although the stage of the complete lab-on-a-chip has not been reached yet, much progress has been made with integrating biomedical assays in microfluidic devices.[17] A more recent development, in which I am personally very interested, is the application of microfluidic technology to understand and control biological

species and processes. The fast growth of this field is due to the combination of a number of factors, which I have already discussed separately in this lecture. *First*, the typical size of microfluidic features is very compatible with that of a biological cell, the basic unit of life. *Second*, microfluidics devices offer ultimate control of fluid flow enabling, for example, the precise setting up of chemical gradients and other micro-environmental factors such as geometry (for example microstructures and surface topography), mechanical cues (stiffness, pressure, deformation) and even electrical signals (by integrating electrodes) that all influence the biological behavior of cells and tissues. *Third*, since microfluidic devices are small and transparent, they are microscopy compatible so that processes within the devices can be observed live. *Fourth*, and crucial, the development of soft lithography and other out-of-cleanroom fabrication methods in particular has made the technology accessible to a broad community of scientists. All these factors combined, have led to unsurpassed possibilities of biological and medical research that were not possible with the conventional tools of biomedical research: petri dishes and animal testing. This merging of technology and biology enables both fundamental studies of biological processes as well as new avenues for understanding health and disease.

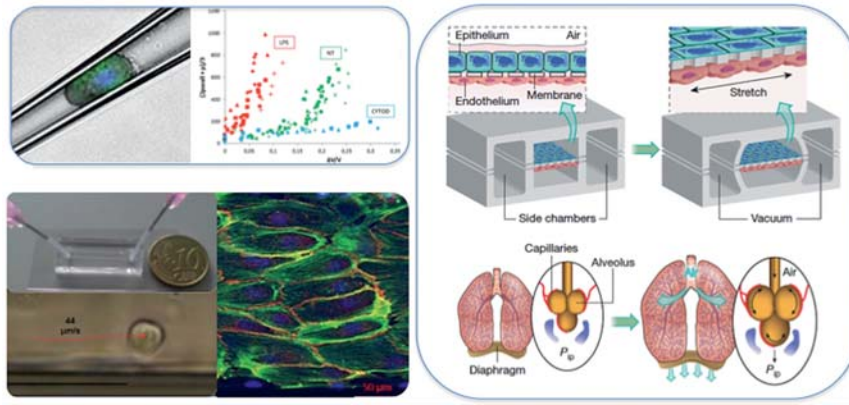

Figure 10

Biological cells and tissues in microfluidic devices. Upper left: A single cell is lodged in a tapered microfluidic channel; from the observed deformation, its full elastic behavior can be characterized.[18] Lower left: an endothelial layer is cultured in a microfluidic channel under varying flow conditions, and the rolling and adhesion behavior of monocytes on this layer is studied.[25] Right: organ-on-chip: the basic unit of the lung is mimicked in this microfluidic device, including the breathing motion.[24]

Microfluidics devices are ideally suited for single cell analysis, and many scientific publications are being published in this area. Being part of the department of Mechanical Engineering, I am interested in the fundamental understanding of the mechanical properties of cells. These are determined by the cell's structure that is formed by the cell wall, the nucleus and the cytoskeleton. The latter consists of an internal network of proteins. Figure 10 (upper left) shows an example of the full stress-strain characterization of a single cell within a tapered microfluidic channel – the deformation of the cell due to the applied pressure is observed directly.[18] Different treatments of the cells affect their cytoskeletal structure, and indeed lead to differences in the observed mechanical behavior. This is not only fundamentally interesting but is also highly relevant for clinical applications. One example of a very important application is the selection and analysis of circulating tumor cells (CTCs) from a blood sample.[19] CTCs are cancer cells originating from a tumor somewhere in the body that have migrated into the blood circulation and travel throughout the body. They may leave the bloodstream at specific locations and seed into an organ, potentially causing a metastatic tumor. The number and the biological characteristics of the CTCs can teach doctors about the progression and nature of (metastatic) cancer, and therefore capturing and analyzing CTCs from blood is extremely valuable and may revolutionize cancer diagnostics. This is a challenging task since CTCs are very rare: there may be just one CTC amongst a billion normal blood cells. Microfluidics approaches are well suited to rise to this challenge.[20] In fact, it has been shown that, at least for some cancers, the combination of size and mechanical properties may be used to distinguish CTCs from normal blood cells, and microfluidics devices can be designed to capture the CTCs from blood.[21]

Beyond single cell analysis, the ultimate merging of microfluidic technology and biology is represented by the very recent development of "organ-on-chip".[22] In short, organ-on-chip devices are microfluidic devices in which, through highly controlled long-term spatial co-culturing of multiple cell types, key structures and functions of human in vivo tissues and organs are recapitulated. Other than the name suggest, it is not full organs that are grown in the devices but the essential features of organs that are representative of the functions as they occur in the human body. Again, the possibility of ultimate control of conditions in microfluidics is crucial for this application. This allows for true mimicking of organ structure and function and enables the study of the interactions between different cell types or tissues while exposing them to their physiologically relevant chemical and physical microenvironment.

Integrated micro-chambers can be viewed as micro-incubators for the 2D or 3D culture of tissues, and micro-channels continuously refresh the culture medium and/or mimic functional blood vessels. The transparency of the chip material allows cells to be visualized and monitored live with a microscope. Using organ-on-chip devices, biological processes can be studied and understood. But this new approach can also revolutionize our knowledge about health and disease, since the approaches currently used are simply not truly representative of human (patho)physiology. *In vitro* culture flasks and dishes do not suffice since the cells and tissues are completely outside of their natural context. And *in vivo* animal models tend not to be representative for human responses. Eventually, organ-on-chip devices may be used as human disease models for developing and testing new drugs and therapies, for example for cancer or cardiovascular and neurodegenerative diseases.[23] This would create a revolution in medical drug development.

The current prototypical example of organ-on-chip is the lung on a chip developed at the Wyss Institute of Harvard University, shown in figure 10.[24] In this chip, the basic unit of the lung, the alveolus, is recapitulated. This consists of two cell layers (an epithelial layer on the “air side” and an endothelial layer representing a blood capillary wall), and by applying pneumatic actuation to the PDMS device, even breathing can be mimicked. This device has been used to study the process of inflammation of the lung and to understand the toxic effect of nanoparticles breathed in.

A final example is shown in the lower left panel of figure 10.[25] This device mimics a blood vessel wall represented by an endothelial layer. The “blood flow” in the device turned out to have a large effect on the structure of the endothelium that formed. The device was also used to study the interaction between immune cells flown through the device, and the endothelium, under both normal and inflammatory conditions.

Research plan

All parts of the story up to now come together in my research plans for the future: out-of-cleanroom technology, active microfluidics, biomimetics and biological applications. Figure 11 depicts the main areas of research for the new Microsystem group at the TU/e Department of Mechanical Engineering. The common theme of all the research carried out in the group is microsystems design and technology using out-of-cleanroom, rapid and flexible micro-fabrication, in particular the unique combination of laser micro-fabrication, soft lithography, printing and mechanical micro-machining. We currently are in the process of building a new state-of-the-art facility for the efficient and flexible manufacturing of microsystems prototypes, *Microfab lab @ TU/e*, which will be a shared TU/e facility accessible to all TU/e departments.

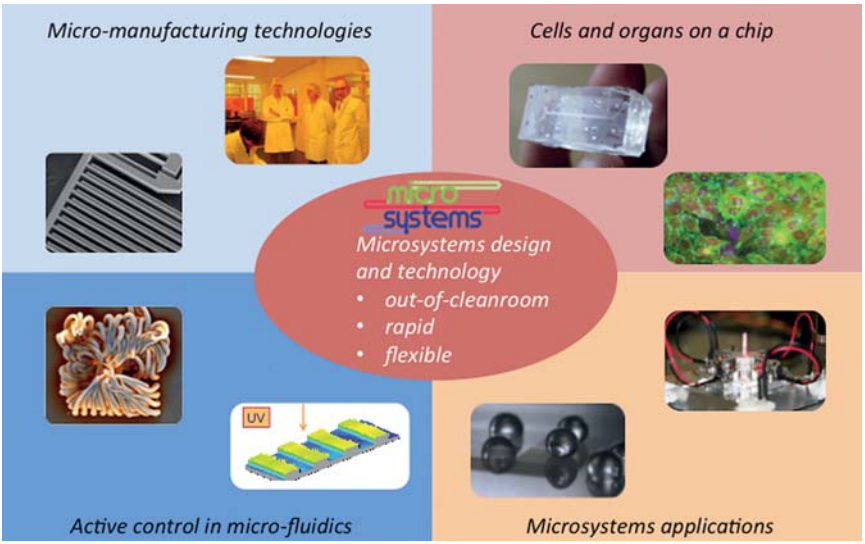

Figure 11
Overview of the Microsystems group.

To achieve active control in microfluidic devices, we will develop, study and apply micro-actuators, responsive surfaces and magnetic bead actuation systems.[26] Biomimetic design will be a valuable source of inspiration for novel concepts. The use of new responsive materials, developed in collaboration with chemical engineering groups, will bring new possibilities for controlling microfluidic flow and mechanical stimuli in the devices.

In collaboration with biological, biomedical, and clinical groups, we will apply our technology to study and understand the behavior of cells, tissues and organs – truly merging technology with biology. This work is aimed at learning about health and disease, and ultimately developing novel therapies and medical drugs. Specifically, we will develop organs on chips as *in-vitro* human disease models, for example to understand cancer metastasis or brain diseases, and we will study the mechanical properties of single cells, with applications such as capturing and analyzing circulating tumor cells.

Microsystems applications can be found in many areas. We will work with industrial partners such as Philips, TNO-Holst Center, NXP and others to develop relevant technologies for these applications, in medical devices, flexible electronics, and advanced sensors and actuators.

Scientific collaborations are very important in all of these activities, especially considering the multi-disciplinary nature of the work. We will actively participate in the TU/e inter-departmental institutes, the Materials Technology Institute (MaTe), the Institute for Complex Molecular Systems (ICMS) as well as with sister research groups at Twente University, Groningen University and Delft University of Technology, and with international institutes such as the Wyss Institute at Harvard University or Ecole Polytechnique Fédérale de Lausanne. For much of the work it is essential to collaborate in research projects with biological, biomedical and clinical groups, as brought together in the Dutch human disease model technologies initiative (hDMT). It is at the interfaces between different disciplines that the most exciting new scientific and technological developments start.

Vision on education

The education of students is one of the main reasons a university exists. It was also an important personal motivation for me to shift from industrial research to academia. I find the contact with these young people, coming from different countries, very rewarding and inspiring: helping them to develop their scientific and personal skills motivates me highly. The central pillar of education at a university is, in my opinion, the direct connection with research. This guarantees that the education is linked to the forefront of science, and students naturally acquire an academic level of thinking and doing.

Students must learn to master a subject field, and be good at it. However, I believe that students at a university should also learn how to position their field within a societal and industrial context, and the educational program should have room for this. Therefore I will stimulate active participation in the educational program of industry, which has a rich presence in the direct environment of TU/e, and direct links with practical applications. All the more since industry is the future working environment for the majority of our students. In this respect, multidisciplinarity is also an important aspect, and our students need to learn to cross over borders to other disciplines. As I have mentioned earlier, it is at these borders that the most exciting new scientific and technological developments start.

I also want to show my students that science can be beautiful, and in many aspects resemble art. Both require and stimulate new ways of looking at the world. Both involve discovering things not seen before, and both follow similar creative processes. And then there is the esthetic nature of a scientific result, which is often as beautiful as a piece of art.

All of this can best be achieved in a small-scale education setting, with short lines between students and teachers, and in my vision this is what TU/e should keep on striving for.[27]

Thanks

Finally, ladies and gentlemen, I would like to thank a number of people.

First of all, scientific research is teamwork, and therefore I express my special thanks to the staff members of the Microsystems group for their enthusiasm to jointly undertake this endeavor: Yves Bellouard, Regina Lüttge, Erik Homburg, Rajesh Mandamparambil, Willie ter Elst and Liesbeth Ballegooij, and all students, PhD students, and postdocs of the group.

Philips Research, where I worked for almost 18 years, has been an incredibly valuable school for me, with a lot of great teachers. I have worked in many different projects on a large variety of topics, and in different roles, with excellent scientists, technologists, and technicians from very diverse disciplines, in a dynamic environment. There couldn't have been a better environment for my personal growth and development. My deepest gratitude goes to all the great people whom I had the privilege to work with, or who supported me in my career. For sure, we will continue working together.

In the past decade, I have had the privilege of being able to combine my main job at Philips Research with a part-time professorship in the Materials Technology Institute MaTe of TU/e, and for sure the great pleasure of working together with the excellent people – both staff members and students – from this group has been a determining factor for my choice to go for a full-time position at TU/e. And, working beyond the boundaries with people from other departments and universities has always been a source of inspiration. Thanks to everybody for that, and let's continue the collaborations! I want to mention one special person: Han Meijer, who hired me in 2004 in his group, and supported me strongly in all the steps I took in my career to get where I am now. Han, thanks!

In the end, I want to thank the most important people. My children, all seven of them, for always keeping me with my feet firmly on the ground. And Wilma, my love, for sharing your life with me.

This concludes my lecture. I thank you for your attention.

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Curriculum vitae

Prof. Jaap den Toonder was appointed full-time professor of Microsystems in the Department of Mechanical Engineering at Eindhoven University of Technology (TU/e) on 1 May, 2013.

Jaap den Toonder (1968) received his Master's degree (cum laude) in Applied Mathematics from Delft University of Technology in 1991 and his PhD degree (cum laude) in Mechanical Engineering from the same university in 1996.

In 1995, he joined Philips Research Laboratories in Eindhoven, the Netherlands. He worked on a wide variety of applications such as optical storage systems, RF MEMS, biomedical devices, polymer MEMS, immersion lithography and microfluidics. In 2008, he became Chief Technologist, leading the R&D program on (micro-)fluidics, and (starting in 2011) materials science and engineering. In addition to his main job at Philips, he was a part-time professor of Microfluidics Technology at Eindhoven University of Technology between 2004 and 2013.

His current main research interests are microfluidics, out-of-cleanroom micro-fabrication technologies, mechanical properties of biological cells and tissues, nature-inspired micro-actuators, and organs on chips.

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