Research Highlights

DOI: 10.1039/b607679n

Molecular sorting of microtubules

The adaptation of molecular motors to efficiently convert chemical into mechanical energy has inspired many researchers in the field of nanotechnology in recent years. The integration of molecular motors in nanoengineered structures opens up the fascinating possibility of manipulating nano-sized materials. For example, a molecular motor such as kinesin translocates along microtubule filaments through hydrolysis of adenosine triphosphate. Such molecular transport systems can potentially be exploited *e.g.* to carry a specific cargo to a designated place.

Recently, Cees Dekker and co-workers from the Delft University of Technology (Netherlands) have managed to integrate kinesin motor proteins in closed submicron channels and to realize active electrical control of the movement of individual microtubule filaments.[1] They fabricated an 800 nm deep channel in a fused-silica substrate (Fig. 1). Kinesin motor proteins and microtubules are added by a pressure-driven flow to flush the necessary protein constituents. The microtubules move along the regions that are coated with kinesin proteins. The speed of $0.75 \pm 0.02\ \mu m\ s^{-1}$ is hereby the same as on a glass coverslip in a standard flow cell. The enclosed geometry of the device confined the microtubules on their track.

The movement of the microtubules can be directed by an electrical field. Since the microtubules are negatively charged, they move towards the positive electrode. Single-microtubule experiments reveal an orientation-dependent electrophoretic mobility and yield a charge of $12 \pm 2\ e^-$ per tubulin dimer under physiological conditions.

This is used to steer individual microtubules into a desired channel of a Y-junction. In front of the Y-junction, an electrical field is induced in a horizontal channel perpendicular to the vertical direction of the motion of the microtubules (Fig. 2). Application of the electrical field results in bending of the leading tip of the microtubules towards the positive electrode. Hence, the movement of microtubules is directed into one of the output channels. Molecular sorting is demonstrated for a population of two differently fluorescent microtubules (stained with (red) rhodamine and (green) fluorescein fluorophors, respectively) on a chip. The differentiation of the microtubules is achieved using a colour-sensitive camera. Red fluorescent microtubules are sent into the red-collecting reservoir, green fluorescent microtubules are sent into the green-collecting reservoir by reversing the polarity of the electrical field. Quantification of the sorting procedure reveals that 72% of the red, and 69% of he green microtubules are directed as intended. The authors propose to improve the sorting method, *e.g.* for purification applications, by automation, which can be incorporated straightforwardly.

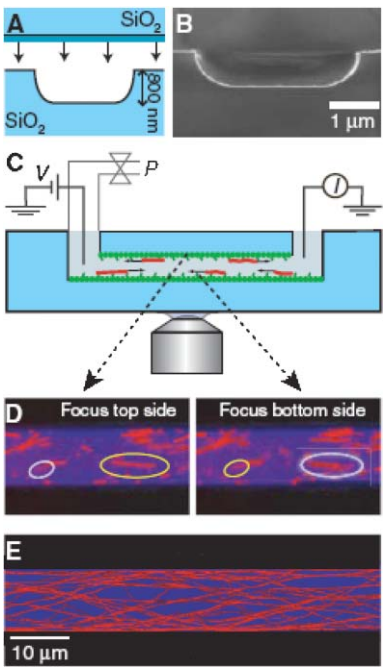

Fig. 1 Movement of microtubules in submicrometer-sized channels. (A) The channels are fabricated in a fused-silica substrate by e-beam lithography and wet etching. They are sealed with a coverslip. (B) Scanning electron microscope image of the channel. (C) Scheme of the channel that is coated with kinesin motor proteins (green), and filled with microtubules (red). Electrical fields can be induced by applying a potential difference between the reservoirs. (D) Using epifluorescence microscopy and adjusting the focus of the objective, movement of microtubules is observed either on the top or the bottom surface of a channel. The image (E) show traces of several microtubules on their way through the channel. The image is integrated during a time period of 7 min. (From ref.1. Reprinted with permission from the American Association for the Advancement of Science.)

Encapsulation of cells, proteins and microbeads

In nature, compartmentalization of biological substances is achieved by vesicles formed by phospholipids that assemble into a membrane. Large vesicles function as cell membranes, whereas small lipid vesicles enable intracellular transport of biomolecules. Lipid vesicles can be synthesized artificially in different sizes, and tailored with different surface properties for various applications. Moreover, encapsulation of particles and substances in lipid vesicles provides a non-toxic, gentle, and efficient way to prepare vesicles for drug delivery, production of biosensors, and engineering of artificial cells. Although a vast number of methods exists to generate lipid vesicles, there are still drawbacks; in particular, the encapsulation efficiency is typically very low. Abraham Phillip Lee and coworkers at the University of California, Irvine, have developed a novel vesicle formation method for encapsulation of biological substances.[2] Encapsulation is achieved in a two-step process (Fig. 3). First, the aqueous liquid containing the target species is emulsified in the lipid phase that consists of dissolved phospholipids in oleic acid. The emulsification process is performed in a crossed microchannel, where the sizes of the emulsion droplets are controlled by the applied flow rate of the lipid phase. In a second step, the emulsion is injected into a water–ethanol mixture. Oleic acid rapidly dissolves in ethanol, which results in the formation of the phospholipid membrane around the emulsions and hence, lipid vesicles are assembled.

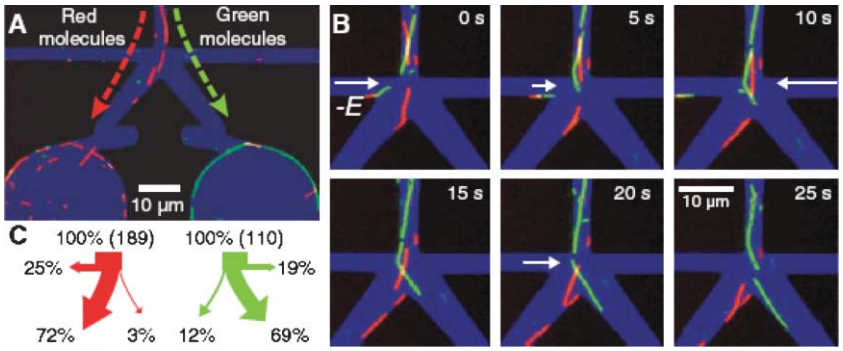

Fig. 2 Sorting of microtubules in a Y-shaped channel. The pictures of red and green labelled microtubules are taken using a color sensitive camera. (A) Microtubules are steered into the desired output channel upon applying an electrical field in the perpendicular channel. (B) Successful sorting events of a green ($t < 10$ s) and a red microtubule. (C) The sorting efficiency is evaluated by counting the initial microtubules (189 red and 110 green, respectively). The majority enters the correct output channel. Minor numbers of microtubules are also steered into the perpendicular "control" channel, or into the wrong output reservoir. (From ref.1. Reprinted with permission from the American Association for the Advancement of Science.)

The green fluorescent protein (GFP) and beads in different numbers can be entrapped inside the lipid vesicles. Live breast cancer cells (MCF7) and Hela cells are also encapsulated (Fig. 4). The cells remain viable throughout the whole process, which was proven by a fluorescent dye staining assay. Since lipid vesicles remain stable for a long time, they might be useful for long term monitoring of single cells. Furthermore, the authors suggest designing cell based bioreactors that allow for the study of single cell to multi-cell interactions inside controlled lipid membranes.

Nanoscale photodetectors

In many applications of microchip technology, the realization of portable, handheld microchip systems is desirable, *e.g.* for point-of-care diagnostics. For stand-alone devices, however, appropriate and miniaturized detection systems

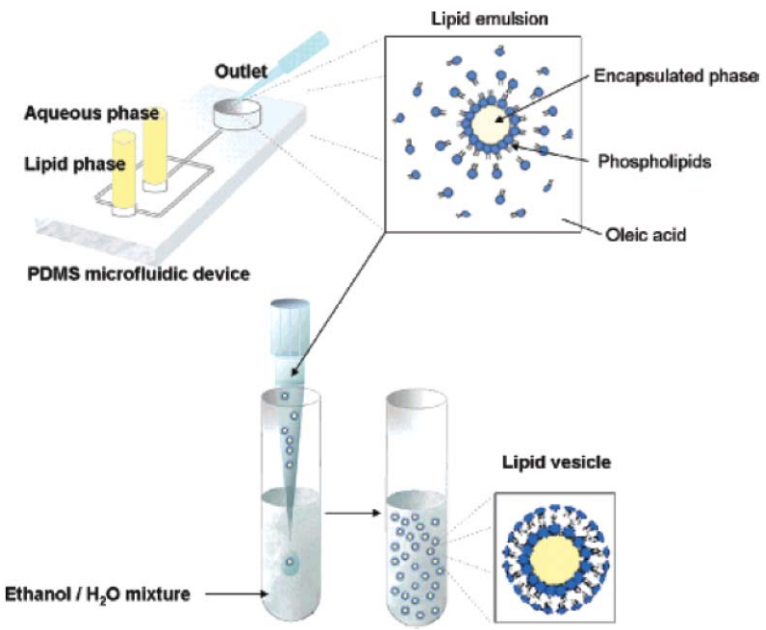

Fig. 3 Formation of lipid vesicles. First, an aqueous solution is emulsified in the lipid phase (phospholipids dissolved in oleic acid). The emulsion is then injected into an ethanol/water mixture, where finally the lipid vesicles are formed. (Reprinted with permission from ref.2. Copyright 2006 American Chemical Society).

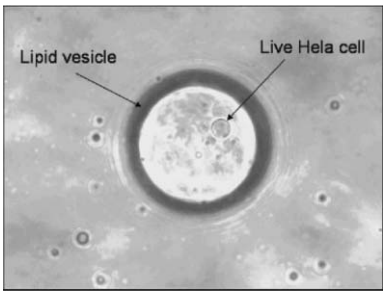

Fig. 4 Picture of an encapsulated Hela cell inside a lipid vesicle. The viability of the cells is verified through a Trypan blue staining assay. (Reprinted with permission from ref.2. Copyright 2006 American Chemical Society).

are required. Since a large number of experiments rely on optical detection, the demand for miniaturized light sources and photodetectors is obvious.

Charles M. Lieber and co-workers fabricated a nanosized photodector that is suitable to be implemented on a microchip. They describe avalanche multiplication of the photocurrent in nanoscale p–n diodes consisting of crossed silicon–cadmium sulfide nanowires.[3] The CdS and Si nanowires with diameters of 80–150 nm and 50 nm, respectively, were grown by a bottom-up approach. The crossed structures were afterwards prepared by microfluidic-directed assembly. On the basis of their former work on nanosized avalanched photodiodes (APDs), the authors have focussed their efforts on cross-nanowire p–n structures in this study, because of the ability to independently tune dopant concentration in the p-silicon nanowires, while a high absorption coefficient with the n-CdS nanowires is maintained. Moreover, abrupt, nanoscale junctions are defined that are ideal for high spatial resolution. Indeed, characterization of the nanoAPDs reveals a spatial resolution of at least 250 nm (the step size used in scanning experiments), and a high gain that can be assigned to the amplification process associated with the p–n diode formed at the crossed-nanowire junction. The dynamic range of the photodetector covers pW to nW with the lowest power of 4 pW that can be detected (corresponding to an estimated detection limit of 75 photons).

One interesting aspect of the crossed-nanowire device is its possible extension to arrays with independently addressable photodetectors. This concept is

demonstrated using an array consisting of two silicon nanowires crossing a single CdS nanowire. Scanning photocurrent data reveal two well-defined photocurrent peaks, while each of the nanoAPDs has characteristics similar to the single APD configuration. The APDs in the array can be independently turned on and off, and no electrical cross-talk is observed when only one detector is exposed to light. This is a first approach towards the realization of dense systems of nanoAPDs that could open up new opportunities for sensing and imaging applications.

Multivortex micromixing

Microfluidic channels are widely used for organic synthesis and biochemical reactions, but it is still a challenging task to find strategies for fast mixing of two or more reactants. Due to the laminar flow regime in microfluidic channels, mixing is dominated by molecular diffusion. As a result, two confluent liquids must typically flow a long distance downstream to become fully intermixed, which often negates many of the benefits of miniaturization.

Much effort was made to increase mixing efficiency in microchannels. Besides the integration of active mixers that rely on external energy, passive mixers were developed. Passive mixers make use of a certain microfluidic channel design, *e.g.* in split-and-recombine strategies.

Arjun P. Sudarsan and Victor M. Ugaz have designed a novel type of micromixer in a topologically simple 2D microchannel. By introducing curvatures and changes in the width of the microchannel, mixing is enhanced due to the combination of two effects. First, so-called Dean vortices arise in the vertical plane of the curved channels resulting from an interplay between inertial, centrifugal, and viscous effects. Second, expansion vortices occur in the horizontal plane due to the abrupt increase in the conduit's cross-sectional area. Two channel designs are described, a planar split-and-recombine micromixer (P-SAR, Fig. 5), and an asymmetric serpentine micromixer (ASM) that consists of a curved channel with sudden changes of the channel width from 100 to 500 μm. The characteristics of the mixing devices are observed by confocal microscopy of aqueous fluorescent dye streams and by determination of binding interactions between intercalating dye and double-stranded DNA. Enhanced mixing is observed for high flow rates. The authors determine a level of 90% mixing in the P-SAR micromixer at a position that is 17.5 mm downstream after the confluence point of two streams, and after the two streams have passed in total four split-and-recombine sections. For the asymmetric mixing device, a level of 80% mixing is achieved at the 7.8 mm downstream position. The beauty of the concept is the two-dimensional design of the mixer, which makes it simple to integrate into any standard microfluidic chip.

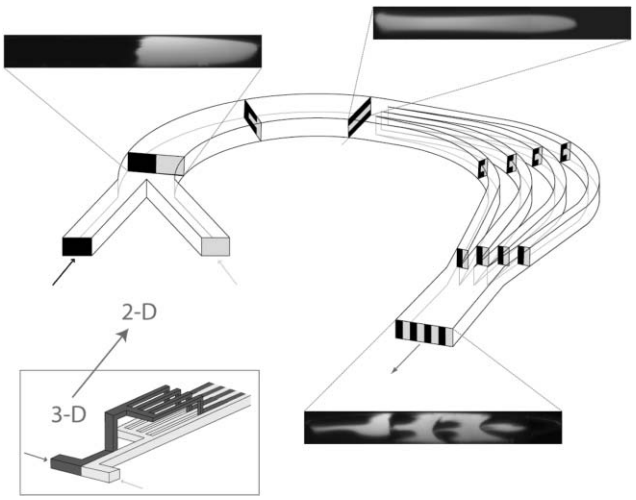

Fig. 5 Curvature induced Dean flow effects can be harnessed to generate alternating lamellae of different fluid species in a planar 2-D microchannel (flow schematics are shown inside the channel, corresponding confocal images are shown outside). When parallel streams of different species travel along a curved flow path, a sequence of transverse rotations can be induced such that a lamellar structure is formed when the streams are rejoined. Inset: example of a conventional 3-D microchannel design to achieve an equivalent lamination effect. (Courtesy of V. M. Ugaz, Texas A&M University, USA).

Petra S. Dittrich
ISAS, Dortmund, Germany
dittrich@ansci.de

References

1 M. G. L. van den Heuvel, M. P. de Graaff and C. Dekker, Molecular Sorting by Electrical Steering of Microtubules in Kinesin-Coated Channels, *Science*, 2006, **312**, 910–914.
2 Y.-C. Tan, K. Hettiarachchi, M. Siu, Y.-R. Pan and A. P. Lee, Controlled Microfluidic Encapsulation of Cells, Proteins, and Microbeads in Lipid Vesicles, *J. Am. Chem. Soc.*, 2006, **128**, 5656–5658.
3 O. Hayden, R. Agarwal and C. M. Lieber, Nanoscale avalanche photodiodes for highly sensitive and spatially resolved photon detection, *Nat. Mater.*, 2006, **5**, 352–356.
4 A. P. Sudarsan and V. M. Ugaz, Multivortex micromixing, *Proc. Natl. Acad. Sci. U.S.A.*, 2006, **103**, 7228–7233.