

# Research Highlights

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## Microfluidic differential manometer

Many studies in microfluidic research focus on the investigation of soft-matter objects such as cells, droplets, foams, vesicles and polymers. The flow and the shape of these deformable objects inside a micrometer sized channel are controlled by the fluid pressure, the viscous stresses, and the particular properties of the objects. The hydrodynamic resistance resulting from the interactions of the soft objects with the fluid is reflected in a dynamical variation of the pressure drop along the microchannel. The ability to measure variations of pressure is therefore a valuable tool to investigate the details of the mechanical properties and structural features of soft-matter objects.

Howard Stone and co-workers at Harvard University have developed a microfluidic device capable of measuring the dynamical pressure-drop variations along a micrometer-sized channel.<sup>1</sup> The microfluidic chip consists of two parallel narrow (5  $\mu\text{m}$  wide and high) channels: a test channel and an identical control (or 'comparator') channel. The channels are merging into a large channel (width of 75  $\mu\text{m}$ ), producing thereby two parallel and adjacent streams of fluid. The liquid flowing through the control channel contains a fluorescent dye to visualize the interface downstream. If an object is placed in the test channel, the flow rate in this channel will decrease, and consequently the pressure drop that occurs in the narrow test section will increase. As a result, the interface downstream is deflected from the original position. Deflections of the interface of a few hundred nanometres are determined, which corresponds to pressure changes of  $\sim 1$  psi, with millisecond resolution. The field of view of the detection camera allows both, the observation of the test channel and of the interface.

With this device, pressure-drop changes due to the passage of cells through the test channels can be measured. Since the dimension of the test channel is smaller than the cells, they are deformed. Any modification of the

membrane stiffness can be evaluated, which can be a biomedical tool for clinical haemorheology and pharmaceutical testing. This is demonstrated by chemical treatment of red blood cells with glutaraldehyde. It results in a higher stiffness of the cell membrane, and thus, the pressure drop is enhanced compared to healthy cells, when the modified cells are entering the test channel. Furthermore, utilizing the microfluidic differential manometer, cell membrane rupture (haemolysis) can be visualized and investigated. As an example in this study, the pressure drop (of  $\sim 1.1$  psi) is determined, when red blood cells block the entrance of the test channel, and haemolysis happens.

The authors emphasize that their measurement technique can be applied to any dynamical process such as chemical reactions and changes of viscosity, by changing the hydrodynamic resistance of the test channel relative to the control channel. It is easy to implement in micro- and nanofluidic systems and naturally applicable to a wide range of soft-matter objects.

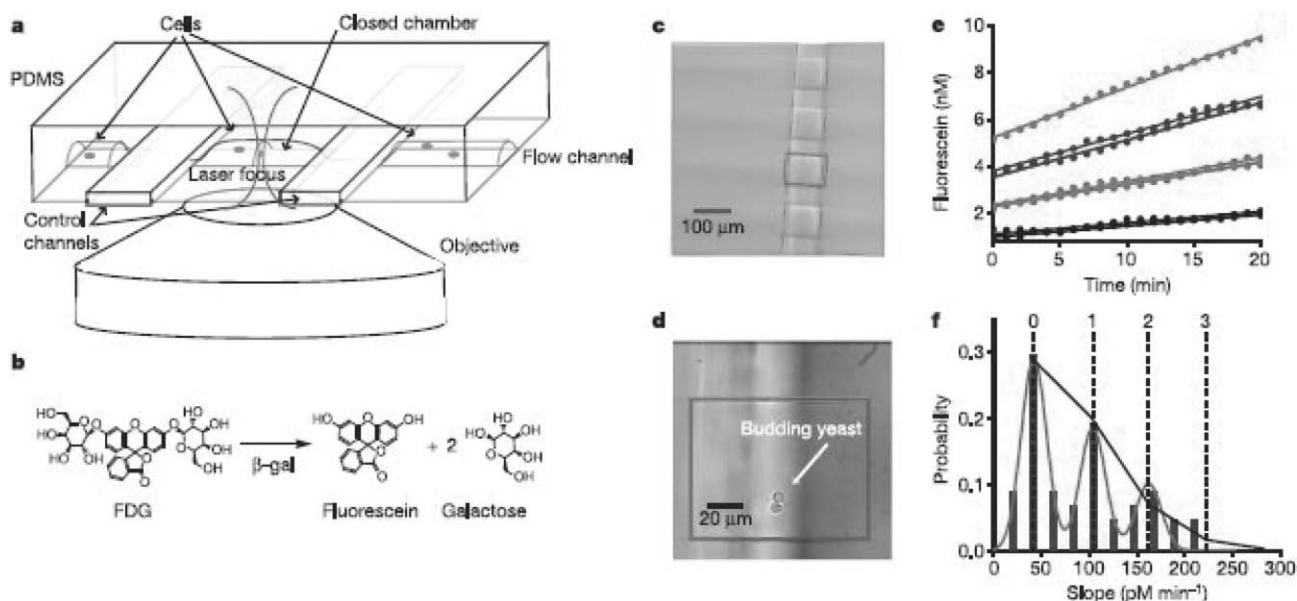
## Single-cell analysis at the single-molecule level

Studies on single-cell level can evaluate heterogeneities of cellular processes with respect to time and cell population. During recent years, microchips have been shown to be a convenient tool for the handling and manipulation of individual cells. Many biological processes in cells, however, happen at levels of low concentration, and the development of single-cell assays in combination with suitable detection techniques is still a critical aspect for single-cell analysis.

Sunney Xie and co-workers demonstrate an enzymatic assay that allows real-time observation of gene expression in living *E. coli* cells and other cell types with single-molecule sensitivity.<sup>2</sup> They utilize  $\beta$ -galactosidase ( $\beta$ -gal) that is a standard reporter for gene expression in both prokaryotic and eukaryotic cells. A single molecule of  $\beta$ -gal can produce a large number of fluorescent product

(fluorescein) by hydrolysing a synthetic fluorogenic substrate (fluorescein-di- $\beta$ -D-galactopyranoside, FDG). This enzymatic amplification has one major drawback, though. The fluorescent products are expelled from the cells rather than retained inside the cells and thus, diffuse away quickly. To circumvent this problem, the authors employ a microfluidic chip fabricated in PDMS by standard microfabrication procedures (Fig. 1). The chip consists of a flow layer that contains the cell suspension, and a layer with control channels. The flow channel can be compressed by two adjacent control channels, so that a closed microfluidic chamber with a volume of 100 pL is formed. Inside the microchambers, cells are trapped and fluorescein expelled from the cells accumulates. The concentration of fluorescein is then determined by fluorescence spectroscopy. A focussed laser beam is used for excitation, and detection is performed by a CCD camera with on-chip amplification. The number of  $\beta$ -gal molecules is calculated by taking the time derivatives of the fluorescence traces and compensating for photobleaching. The microchip is mounted on a motorized stage of an inverted microscope, and up to 100 microchambers are scanned within 2 minutes.

First, the ability to detect single  $\beta$ -gal enzymes is shown. A mixture of the enzyme and the substrate FDG is injected into the microchambers and the fluorescence traces are recorded. The distribution of the hydrolysis rates derived from the slopes of the fluorescence traces shows quantized and evenly spaced peaks, which are attributed to integer numbers of the  $\beta$ -gal enzyme. The same procedure is applied to determine the number of  $\beta$ -gal enzyme expressed in *E. coli* bacterial cells. A step-wise increase in the hydrolysis rates, indicating a stochastic burst-like expression of new  $\beta$ -gal molecules, is observed for dividing cells under conditions where gene expression is highly repressed. Beside this temporal stochastic gene expression in a single cell, a distribution of protein number in a population of



**Fig. 1** Single-cell analysis on a microchip: (a) The microchip consists of two layers. A microchamber is formed in the flow channel by actuating the control channels. (b) Enzymatic amplification: hydrolysis of the synthetic substrate FDG by the enzyme  $\beta$ -gal yields the fluorescent product (fluorescein). (c) and (d) are DIC images of the microchambers, and budding yeast cells entrapped in a microchamber, respectively. (e) Hydrolysis of FDG by purified  $\beta$ -gal in a microchamber. The concentration of fluorescein increases with time. The different slopes are reflecting different numbers of the enzyme  $\beta$ -gal. (f) A histogram derived from the slopes in (e). The peaks can be assigned to discrete numbers of the enzyme. (Adapted by permission from Macmillan Publishers Ltd: Nature,<sup>2</sup> copyright 2006.)

cells at a particular time is observed. The authors create a model to explain their observations and suggest two different regimes of stochasticity in protein expression.

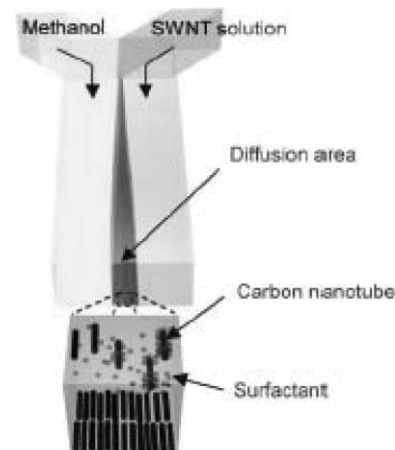
The microfluidic single-cell assay is applicable to different prokaryotic and eukaryotic cell types expressing  $\beta$ -gal as a reporter. It opens up possibilities for system-wide characterization of the expression of these low copy number proteins. Moreover, the simple microchip design allows a dense package of the microchambers on a chip and thereby high parallelization of single-cell observation.

## Patterning of carbon nanotubes

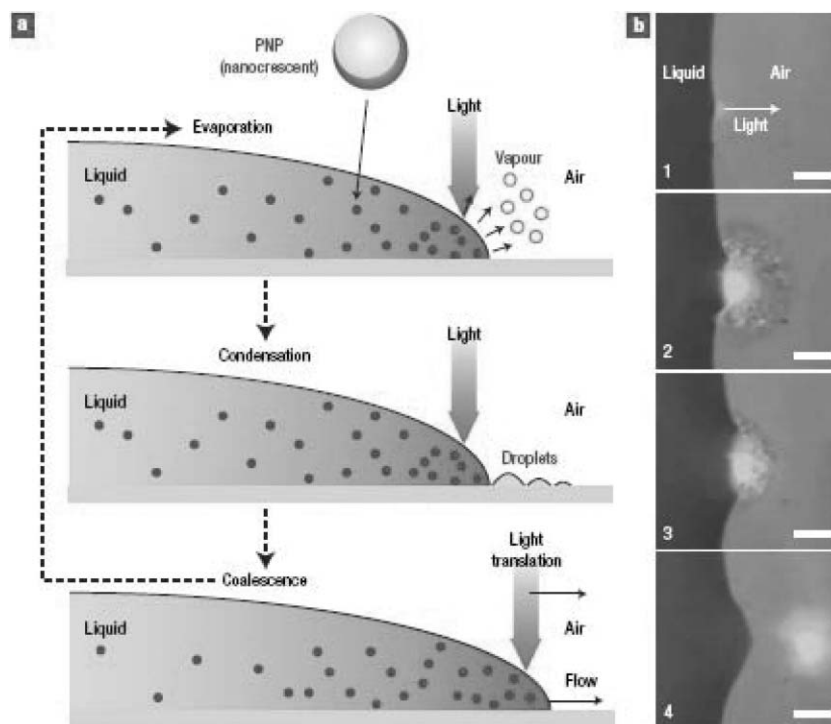
Single-walled carbon nanotubes (SWNTs) exhibit remarkable electrical, mechanical, and chemical properties, which makes them potentially useful as semiconducting or conducting elements in sensors, transistors, and other electronic systems. The integration of SWNTs into electronic circuits, however, is challenging, and requires techniques for depositing and patterning of individual SWNTs, or SWNT aggregates, from solutions onto substrates.

Park *et al.* have addressed this problem by exploiting the laminar flow inside a microfluidic device for controlled patterning and flocculation of SWNTs onto a wide range of substrates, including plastics, without the need to functionalize the substrates or the tubes with chemistries designed to create strong interactions.<sup>3</sup> In this approach, two streams—methanol and an aqueous suspension of surfactant-stabilized SWNTs—are joined at a microfluidic Y-junction (Fig. 2). Methanol exhibits a strong affinity for the surfactant and hence, the concentration of the surfactant in the aqueous stream is lowered. Since the flow is laminar, only diffusive mixing occurs near the liquid–liquid interface. When the concentration of surfactant available to interact with the SWNTs is reduced to values below the critical micellar concentration, the tubes are no longer well suspended. Interactions between SWNTs can now result in formation of bundles, and interactions of SWNTs to surfaces can lead to coating of these surfaces. The flow duration determines the coverage of the deposited SWNTs, which is determined by AFM. High shear forces generated by high flow rates improve the degree of alignment of the nanotubes.

Dynamical changes of flow rates during the deposition sweep the interface of the methanol and water streams across the channel to generate wide SWNT stripes or multiple, parallel narrow stripes. Since the microfluidic channels are fabricated in flexible PDMS, curved and uneven surfaces, *e.g.* the surface of spherical lenses, can be coated with this technique.



**Fig. 2** A microfluidic Y-junction for patterning of single-walled carbon nanotubes by multiphase laminar flow and controlled flocculation. (Adapted with permission. Copyright 2006, Wiley-VCH.)



**Fig. 3** (a) The principle of optofluidic control using photothermal nanoparticles (PNPs, for explanation: see text). (b) Video prints showing the light-driven advance of the liquid–air interface of a 1 nM PNP water solution on a glass surface. (Adapted by permission from Macmillan Publishers Ltd: Nature Materials,<sup>4</sup> copyright 2006.)

To demonstrate the ability to build electronic devices with patterned SWNTs, organic thin film transistors are fabricated, in which SWNT networks serve as source/drain electrodes. The electrical behaviour ranges from insulating for low SWNT coverage, to semi-conducting for medium SWNT coverage, and to metallic for high coverage, and is modulated by controlling the flow duration.

### Optofluidic control in microchannels

The control of liquid transport, *i.e.* pumping and guiding of fluids, is obviously crucial in microfluidic systems. In most applications, the driving force for liquid flow is a pressure gradient or an electrical field, which is typically realized by use of syringe pumps and valves, or by integration of electrodes, respectively.

Luke Lee and co-workers at the University of California at Berkeley have recently presented a novel method to drive and guide liquids, which is based on a direct optical-to-hydrodynamic energy conversion utilizing photothermal particles and laser light.<sup>4</sup> The all

optical-control of fluid movement does not require any additional functionality such as mechanical valves or external pumping devices.

The principle is shown in Fig. 3. Gold nanocrescent particles with a strong absorption band around 780 nm were introduced to an aqueous solution in a concentration of  $\sim 10^{14}$  particles  $l^{-1}$  ( $\sim 1$  nM). The nanoparticles absorb light that is focussed in proximity of the liquid–air interface. The subsequent temperature increase around the nanoparticles results in evaporation of the water at the liquid–air interface. The vapour condenses into droplets in front of the interface. The droplets coalesce with the original bulk liquid, and the liquid–air interface advances. Continuous fluid movement is facilitated if the processes are repeated as the laser light is translated. The flow speed in a linear microchannel achieved by this technique depends on the power (here up to 20 mW) of the focused laser light as well as on the concentration of the nanoparticles. A velocity of  $500 \mu\text{m s}^{-1}$  is experimentally realized in a  $10 \mu\text{m}$  wide channel. The maximum speed, however, is limited due to time that is needed for droplet formation, growth

and coalescence with the bulk liquid, and a theoretical value of  $\sim 1 \text{ mm s}^{-1}$  is evaluated by the authors. They also demonstrate optofluidic control in more complex systems. The guidance of liquids at two adjacent T-shaped channel junction is presented, as well as mixing of three separate liquid streams into one. The simultaneous movement of fluids in parallel microchannels is feasible by use of a focused laser line that exposes each microchannel with a small portion of light (here smaller than 1 mW per microchannel). The rise of temperature up to  $70^\circ\text{C}$  is highly localized to the illuminated spot, which is confirmed by thermo-fluorescence microscopy and thermochromic microcapsules. Hence, biomolecules or living cells suspended in the aqueous buffer solution are not affected.

Beside the use of optofluidic control in complex microsystems for biomolecular and cellular medicine, the authors expect applicability of the technique for optically powered machines, such as micro-scale power systems or solar heating systems.

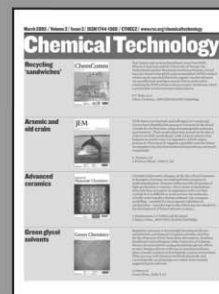
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