Research Highlights

Centrifugal microdevices

Chip-based analysis systems provide for efficiency gains with respect to analytical performance, analysis times, throughput and cost, however the functional integration of sample handling and sample pre-treatment upstream of microscale reaction and separation units is key to defining the potential applications of such instruments. Centrifugation is a well-established sample pre-treatment procedure commonly used to separate, discriminate or concentrate materials suspended in a liquid medium. The efficient functioning of the technique relies on the varying response to gravitational fields of molecules or macromolecules of differing sizes in suspension. Macroscale centrifuges typically generate high centrifugal forces by mechanically spinning carrier vessels at high rotation speeds. Unfortunately, such approaches are ill suited to transferal to chip-based formats and thus alternative routes are desirable. To this end, Daniel Chiu and colleagues at the University of Washington have recently reported the creation of centrifugal microdevices containing no moving parts. These microfluidic devices incorporate a diamond or trapezoidal notch along the sidewall of a straight microchannel, which causes flow detachment at the notch opening. Variation in fluid flow along the primary microchannel is then used to generate radial accelerations within the notch cavity. To demonstrate the efficacy of this approach the authors use microdevices incorporating a diamond shaped notch to separate fluorescent polystyrene and silica beads of different density. As the flow velocity in the primary microchannel is increased from 1.5 to 20 m s^{-1} polystyrene beads are concentrated towards the center of the vortex in the notch, whilst the silica beads (of high density) are driven towards the chamber edge. In addition, similar devices incorporating a trapezoidal notch are shown to generate radial accelerations as high as $10^6 g$. The efficiency with which high centrifugal forces can be generated in relatively simple microfluidic circuits should prove useful for integration with other unit operations (such as separations and reactions) in microfluidic devices.

Nature, 2003, **38**, 425.

High-throughput cell analysis

Rapid analysis of single cells is important in a wide range of biological and medical applications due to variations in individual cell composition and activity. Indeed, the ability to measure the chemical composition of single cells provides a direct route to the determination of abnormalities and thus identification of disease states. Not surprisingly, a number of techniques have been developed to probe single cells. Of these electrophoretic techniques have proved popular due to fast analysis times, efficient separation of cellular material and low achievable detection limits. Although successful, most methods only provide for analysis rates of a few tens of cells per day. Michael Ramsey and co-workers at Oak Ridge National Laboratories have addressed this problem by creating a microfluidic device that integrates cell handling, fast cell lysis, electrophoretic separation and detection of fluorescent cytosolic dyes. Crucial to operation is the ability to rapidly lyse cells prior to electrophoresis. The Oak Ridge group achieve this using electrical fields, where application of ac electric fields under appropriate conditions allows cell lysis to occur within 33 ms. Importantly, adhesion of intact cells and cellular debris is inhibited throughout the fluidic network by using a hydrophilic dynamic coating agent. Once cell lysis has been performed, the hydrolysed dyes in the cell lysate are then automatically injected into a separation channel and detected within a couple of seconds. Using this approach the authors demonstrate analysis of Jurkat cells at rates of between 7 and 12 cells per min, representing a significant increase in cell throughput when compared to conventional electrophoretic analysis. The combination of fast analysis times and integrated cell processing operations provides the basis of a unique clinical tool allowing facile analysis of cell populations for disease diagnosis.

Analytical Chemistry, 2003, **75**, ASAP article.

Screening protein crystallization conditions

Extraction of biological meaning and function out of the raw sequence data from genome projects is crucial in the post-genomic era. Of particular interest is the relationship between the primary and secondary structure of proteins and their three-dimensional form and function. The controlled growth of high quality crystals (for X-ray diffraction analysis), although important is extremely difficult due to its dependence on factors such as temperature, pH, concentration and impurity levels. Consequently, this process is recognized as the primary bottleneck in structure determination.

Determination of ideal conditions for crystallization is normally achieved through manual screening of protein solutions. This process is both time-consuming and reagent intensive. Since the key to such screening operations is in the ability to deliver and mix precisely metered volumes of reagents, and provide appropriate conditions for crystallization, the use of microfluidic systems is highly beneficial. Rustem Ismagilov and colleagues at the University of Chicago have recently described a highly novel microfluidic system for just this purpose.

The simple microdevice is fabricated in PDMS and contains five input channels. Four of these are used to introduce the aqueous reagents that form the crystallization medium and the other is used to introduce a water immiscible oil. The aqueous streams meet at the entrance to the flowing oil stream where they spontaneously form a droplet (surrounded by the oil phase). Since the volume of each aqueous solution injected is proportional to the volumetric flow rate at which it is introduced, the relative amounts of each reagent in the droplet can be systematically varied by changing the relative flow rates of the aqueous input streams. After loading the device with droplets flow is terminated and the device incubated to initiate crystal formation. Droplets are monitored optically through the PDMS substrate and evaporation of the aqueous phase is controlled.

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Using this approach the authors demonstrate reproducible crystals for thaumatin, bovine liver catalase and glucose isomerase under precise conditions (Fig. 1). In addition, evaporation is shown to yield additional conditions that facilitate crystallization. Importantly, the screening system allows automated generation of hundreds of individual crystallization environments within a couple of minutes and uses only 4 nL of protein solution per screen. The Chicago team is currently developing crystal harvesting and processing techniques for more comprehensive crystallization studies.

Journal of the American Chemical Society, 2003, **125**, 11170–11171.

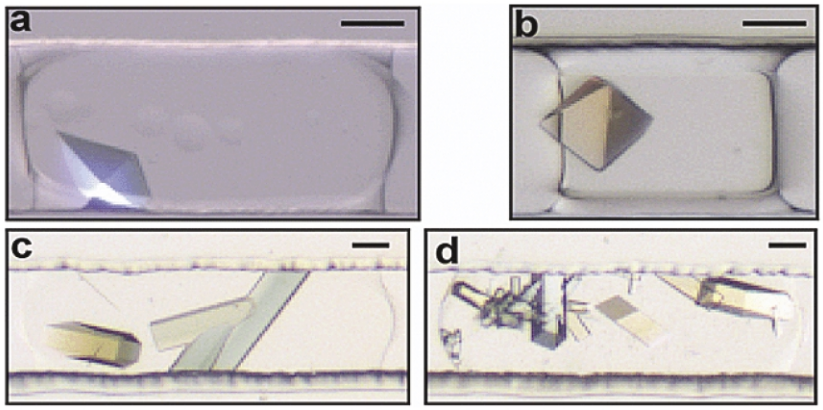

Fig. 1 Micrographs of protein crystals obtained inside droplets on a microfluidic chip. Each scale bar is 50 microns. (a, b) Thaumatin, (c) bovine liver catalase, and (d) glucose isomerase. The crystal in (b) was obtained by allowing partial evaporation of droplets. (Adapted with permission. Copyright 2003, The American Chemical Society.)

Microfluidic electronics

Organic materials are poised to transform the world of circuit and display technology. Organic electronic components are low-cost, easy to fabricate and compatible with polymeric or plastic materials. These characteristics also suggest easy integration with microfluidic systems to create novel electronic components or highly integrated and miniaturized microfluidic devices. John Rogers and associates at the University of Illinois at Urbana-Champagne and Lucent Technologies have reported the fabrication of a novel type of transistor that combines a conducting microfluidic source and drain electrodes with a thin film *p*-type organic semiconductor (Fig. 2). The device consists of a structured PDMS top plate containing two reservoirs and two separate microchannels. The region where the channels are parallel is defined as the transistor region and contacts a thin film of pentacene on the bottom substrate. Reservoirs are filled with mercury and application of mechanical pressure allows mercury to be pumped along the channels. As the mercury flows over the pentacene pad it completes the transistor whose source and drain electrodes are defined by the separation of the two microfluidic channels and the length that the fluids are collinear. The authors demonstrate low contact resistances between mercury and pentacene and high operational performance. More importantly reversible and systematic tuning of the devices in a variety of situations is also achievable. Such electronic devices will almost certainly play an important role in organic electronic and microfluidic applications due to their simple fabrication, good performance characteristics and configurational flexibility.

Applied Physics Letters, 2003, **83**, 2067–2069.

Microfluidic mixing and freeze-quenching

A desirable benefit when using continuous flow systems is the ability to probe the temporal evolution of an event, such as a chemical reaction or a conformational relaxation, by initiation of the event at a given point in space and subsequent analysis as a function of distance downstream. This general concept when combined with the control aspects of microfluidics is ideal for monitoring short-lived intermediates along a reaction coordinate. Conventional approaches to intermediate analysis are hampered by mixing dead times of a few milliseconds. Consequently, a number of microfabricated mixing devices have been developed to reduce dead times to a few microseconds. A recent example of such an approach has been described by Syun-Ru Yeh and co-

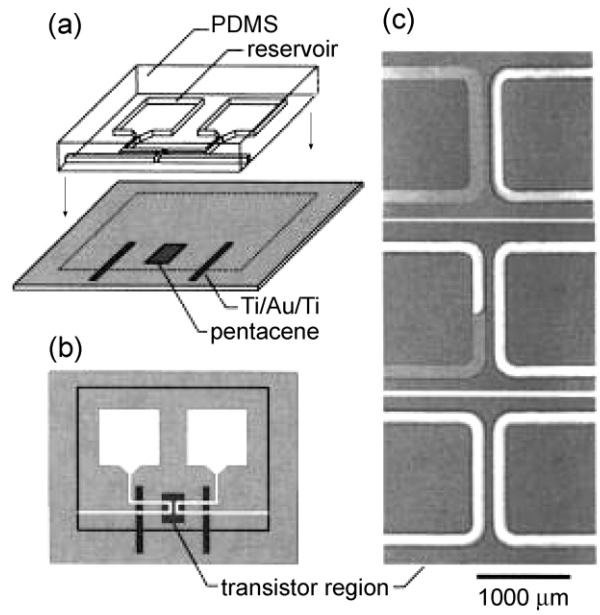

Fig. 2 (a) A schematic view of a PDMS element with relief on its surface (top) and a substrate that supports the semiconductor, gate dielectric, Ti/Au/Ti contact lines, and gate electrode. (b) A schematic illustration of the assembled device. (c) Optical micrographs of the transistor region with mercury (white) pumped into the channel on the left hand side to various degrees. (Adapted with permission. Copyright © 2003 American Institute of Physics.)

workers at the Albert Einstein College of Medicine, New York. They describe the use of a silicon micromixer interfaced with a freeze-quenching device to characterize the binding of sodium azide to myoglobin.

The microfluidic mixer operates by introducing two fluid streams into a 450 pL mixing chamber containing seven vertical pillars (10 μm diameter). These pillars act to disrupt fluid flow and ensure rapid mixing of the two streams. The output stream exits the mixer as a jet with a velocity of 20 m s^{-1} and is directed towards the macroscale freeze-quenching device. This consists of two liquid nitrogen-cooled copper wheels that revolve in opposite directions. When the fluid jet hits the wheels it instantaneously freezes, is ground into fine powder and is deposited into a collection tube. Actuators control the distance between the mixer and the drive wheel and thus determine the reaction time probed (down to 50 μs). The authors demonstrate the efficacy of the system by monitoring the binding of azide to myoglobin at different temperatures. Studies revealed the generation of powder particles of good spectroscopic quality, thus allowing simple extraction of kinetic data from resonance Raman and EPR measurements. Furthermore, since the powder is collected prior to analysis other spectroscopic measurements can be made if needed. This general approach will be particularly useful for studying many biological systems since it preserves room temperature phases of biological systems in the frozen solution over a wide temporal window.

Analytical Chemistry, 2003, **75**, ASAP article.

Video-speed electronic paper

The ability to combine the ergonomically pleasing characteristics of paper and the real-time manipulation of data afforded by electronic displays to create so-called ‘electronic papers’ has been the focus of much interest over the past few years. A number of different approaches have been mooted and demonstrated but common problems include inadequate robustness and slow response speeds. Robert Hayes and Johann Feenstra at Philips Research Eindhoven have recently described a new kind of electronic paper, based on the principle of electrowetting, which provides for a video-speed display.

The idea behind such a system involves the controlled manipulation of a coloured oil film adjacent to white substrate. Specifically, if an oil film is confined between an insulated electrode and an aqueous phase, application of a voltage between the electrode and the aqueous phase can be used to move and displace the oil from over the electrode in certain positions. When no voltage is applied, the colored oil forms a flat film between the water and an insulating coating of an electrode, resulting in a coloured pixel. When a voltage is applied between the electrode and the water, the interfacial tension between the water and the insulating coating changes, causing the water to move the oil aside. This results in a partly transparent pixel, or a white pixel if a reflective white substrate is used.

The authors realize this idea using a white polymer foil (as the substrate), a 15 nm ITO film (as the transparent electrode), non polar dyes in alkanes (as oil films) and a fluoropolymer (as the hydrophobic insulator). Initial studies yield reflectivity and contrast values that are comparable to electrophoretic displays and close to the optical performance of conventional paper. Importantly, the authors also show that in small pixel elements response times of approximately 10 ms are achievable, allowing visualization of video content. This is due to efficient capillary forces that act to reform oil films after application of a voltage. Although the functioning of such displays depends on liquids spreading over surfaces, the ‘papers’ produced are not effected by tipping since fluids are held in place by capillary forces that dominate gravity and other mechanical forces. The technology is particularly attractive for electronic-paper applications, for which high-brightness and contrast-rich reflective displays are desirable, and should also find use in other display applications requiring full colour and video speed capabilities.

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