Future lab-on-a-chip technologies for interrogating individual molecules

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Advances in technology have allowed chemical sampling with high spatial resolution and the manipulation and measurement of individual molecules. Adaptation of these approaches to lab-on-a-chip formats is providing a new class of research tools for the investigation of biochemistry and life processes.

A range of powerful technologies exists for observing and identifying individual molecules. Well-established forms of microscopy such as electron microscopy and optical fluorescence microscopy have allowed us to image and, with the appropriate labels, identify the chemical nature of individual molecules. More recently, scanning probe microscopes have expanded the possibilities for observing and interacting directly with individual molecules. The fine probe of a scanning system can localize electrical measurements to a selected molecule, or measure the mechanical properties of a single biopolymer. Modern material-processing technologies, analogous to those that have been so successful in converting electronics to a 'chip-based' technology, are being explored for possible chemical or biological research lab-on-a-chip approaches[1].

Interest in scaled-down analytical processes, combined with advances in microfluidics, is motivating various chip-based methods in which analyses can be carried out more rapidly and at lower cost via small-scale systems than with current laboratory bench-scale methods. The new research approaches discussed below are motivated by the possibility of observing new phenomena or obtaining more detailed information from biologically active systems. For this, a class of research systems is evolving that uses a range of new physical and chemical approaches to biomolecular analysis. The complexity of life processes and the richness of molecular biology provide fertile ground for research with these new lab-on-a-chip approaches. The development of these new chip-based technologies is changing the nature of the questions that we can ask and for which we can seek experimental answers at the molecular level.

The developing chips are formed using technologies for inorganic-device processing combined with synthetic chemistry and biochemistry. The chips are beginning to integrate electrical, optical and physical measurements with fluid handling to create a new class of functional chip-based systems. The systems provide spatial localization that is relevant to observing, for example, the function of a single active enzyme, the activity of a single receptor on a cell surface, or the release of molecules from a single vesicle exocytotic event in an immune-system cell. A long-term result of this research could be a new class of fluid-handling chip systems engineered to use or analyse individual molecules. This could form the basis for ultra-sensitive sensors and medical diagnostic systems.

Molecular imaging and probes

The scanning probe instruments, such as the atomic force microscope and the scanning tunnelling microscope, have allowed the execution of a class of experiments in which individual molecules can be manipulated and probed. They are providing insights into biomolecular systems previously addressed only by indirect investigations. Scanning tunnelling microscopy can image molecules on surfaces and directly observe their conformation and structure[2,3]. Atomic force microscopy (AFM) can image molecules in a natural hydrated condition and capture images while the molecules are functioning[4–6]. The AFM has also been used to observe the folding and unfolding of individual protein molecules[7–9]. Observing the function of active molecules in a condition as close as possible to *in vivo* circumstances can provide new insights into how living systems function at the molecular level.

Ashkin was the first to use gradient forces from optical beams to trap particles and exert controlled forces on small beads, viruses and bacteria[10,11]. The technique, which came to be known as 'optical tweezers', proved to be a powerful tool for applying controlled forces to individual molecules[12–15]. Typical experiments involve chemically binding a molecule of interest to a bead and chemically linking the other end of the molecule to a surface or another bead. The optical tweezers can be used in fluids and, as a result, can be used for biomolecules in a natural hydrated and functioning condition. This permits force–distance measurements as a biopolymer is unfolded, or the investigation of the breaking of bonds in specific reactions. This technique is now widely used in biophysical studies of single molecules. It has done a great deal to move researchers towards thinking of single molecules as accessible objects that can be selected and studied for their individual properties. It has also helped motivate chip-based approaches for individual-molecule analysis.

Patch-clamp technology, another form of localized electrical probe, revolutionized our ability to study active ion channels in cell membranes[16]. The technology uses a glass pipette drawn down to form a micrometre-scale aperture that can make a tight, electrically insulating seal to a cell membrane. With the electrical current conducted and measured through the pipette, electrical charge is constrained to conducting paths inside the sealed region of the pipette tip. This was a revolutionary technique that allowed the observation of single-molecule ion channels in living cell membranes.

Although powerful and productively used in experimental research, the single probe methods use tedious manipulation of the individual probes and do not take advantage of the integration, parallelism and automation available from a chip-based system. They do, however, set the stage for migration of these approaches to chip-based methods, and possibly further integration into research systems. Researchers are beginning to develop and use engineered devices that are often fabricated in large arrays for accessing individual molecules.

Developing technologies for lab-on-a-chip integration

Technologies exist that unite lithographic approaches for processing hard materials with soft material processing, fluidics and biochemical patterning. These technologies, which are related to those used in commercial device manufacturing, reliably produce systems with feature

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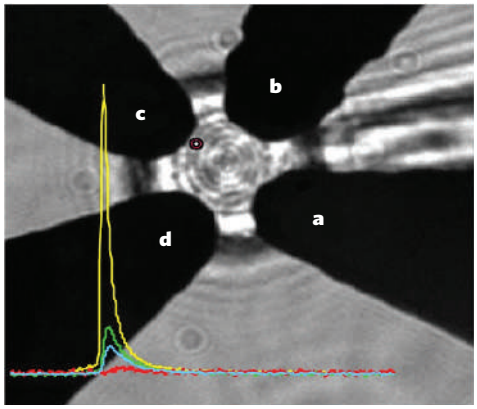

Figure 1 | Optical micrograph of a 4-electrode electrochemical detector array on which a chromaffin cell has been placed. The superimposed trace is the measured current as a function of time at electrodes **a**–**d** (**a**, red; **b**, green; **c**, yellow; **d**, blue). The small circle drawn near electrode **c** indicates the location of the fusion pore opening, calculated from the relative current collected from the four electrodes[41].

sizes of less than 100 nm[17–22]. They are also used to create complex and massively parallel electronic devices, creating billions of essentially identical devices in a chip format. Optical sources and detectors have also been miniaturized and made parallel, and are available in consumer products such as video cameras and displays. This is a powerful and well-developed technology that can be exploited in the research arena today, and possibly in the future in a new class of highly-functional chip-like devices for biochemical analysis.

At this stage of research, many different materials are being explored and used. Polymers are attractive for many microfluidic uses because they are easily manufactured by embossing or moulding[23]. They can also be bonded to other surfaces and are therefore often used to form a fluid channel on another surface, such as silicon, on which an electronic device has been formed. Single-molecule approaches using electrical or optical detection may create other material requirements with regard to electrical or optical properties. Semiconductors and metals are obviously necessary components of electrical detection schemes. Semiconductor nanowires and carbon nanotubes, for example, are being studied as sensor components[24–26]. Mechanical devices are also being explored and integrated in fluid systems[27–30]. In addition, porous media for sample concentration and filtration can be formed in fluid channels[31]. The situation is nothing like that of silicon microelectronics technology, in which the technology is mature and material technologies are monolithic. However, some common materials and processing techniques are evolving, often on the basis of silicon compounds and polymers, that suggest the possibility for greater integration of fluid handling with optical, electrical and mechanical devices.

Electrical chips with high spatial resolution

The development of the planar patch clamp is an example of the conversion of a very successful probe technique to a chip-type device[32–39]. In this approach, rather than using the narrow aperture of a drawn glass pipette, an electrical probe is formed by an etched opening, of the order of a micrometre, that can be readily fabricated in a thin membrane formed on a planar surface. The macroscopic electrodes are then placed on opposite sides of the aperture, with all current forced to traverse the narrow aperture. The entity of interest, typically an ion channel in a lipid layer spanning the aperture, is thus electrically isolated. Schmidt, Mayer and Vogel[32] demonstrated such a device in a silicon nitride membrane supported on an etched silicon wafer, used to observe the activity of a single voltage-gated ion channel of the peptide alamethicin integrated in a lipid bilayer. The use of a fabrication approach with a lithographically defined aperture formed a reliable aperture size, and opened up the possibility of making arrays of many apertures for localized electrochemical measurement. Although they do not replace the traditional moveable probe technique, microfabricated planar patch-clamp-like devices such as this are being used. Their planar geometry allows them to be engineered into sensor configurations.

Electronic-chip-fabrication technology can be used to create arrays of electrodes for probing discrete electrical events in living systems. Lindau's group, for example, described an electrode array for spatio-temporal resolution of single exocytotic events in living cells[40,41]. Using a patterned metallic electrode array, on which the cells were placed, electrochemical signals were recorded from the oxidation of catecholamines released by the cell during events in which a vesicle contained in the cell fused with the cell membrane, releasing its contents. By measuring the relative strengths of the current signals from each electrode, the researchers could calculate the position of each individual exocytotic event as well as the total quantity of ions released and the time taken by the event. This level of quantitative detail is difficult to obtain by other methods and, again, the format allows for scaling the number of groups of identical electrodes for measurements, in principal, on large arrays of cells. This is of interest for a number of basic cell-biology studies, and is also of potential utility in obtaining more detailed information about the effects of potential drugs on cellular function.

Control of the structure and design of the arrays also enables integration with complementary measurement approaches. With the same types of electrode array (Fig. 1), Lindau and colleagues were also able to record simultaneously fluorescence images of fusion-pore events in the cells. This brought to bear the full capability of chemically selective fluorescent dyes and optical imaging to verify the interpretation of the electrochemical imaging. This also indicates the value of this type of lab-on-a-chip in terms of engineering the planar devices for integration for multiple analytical processes.

Of course, the capabilities of integrated electronics extend well beyond those of passive electrodes; researchers can incorporate active electronic devices to allow more complex functions, such as signal amplification, to be carried out. Many groups have used microfabricated electrode arrays for extracellular recording of action potentials from

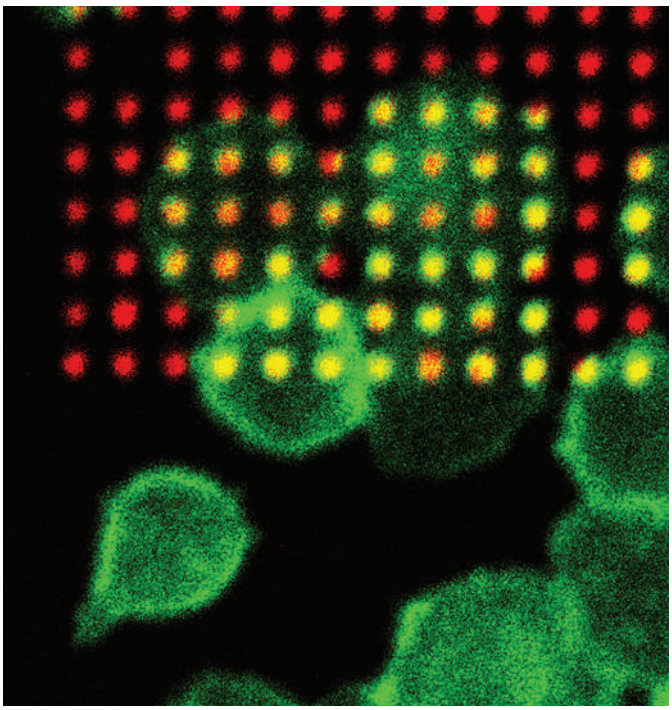

Figure 2 | Fluorescence micrograph of micrometre-size patterned lipid bilayers containing specific ligands used to cluster mast-cell receptors. Clustered receptors (green) stimulate transmembrane signalling events, and the patterns (red) allow visualization of spatial regulation of reorganizing cellular components. Co-concentrated red and green fluorescence appears yellow[45,46]. (Image courtesy of B. Baird, Cornell University, USA.)

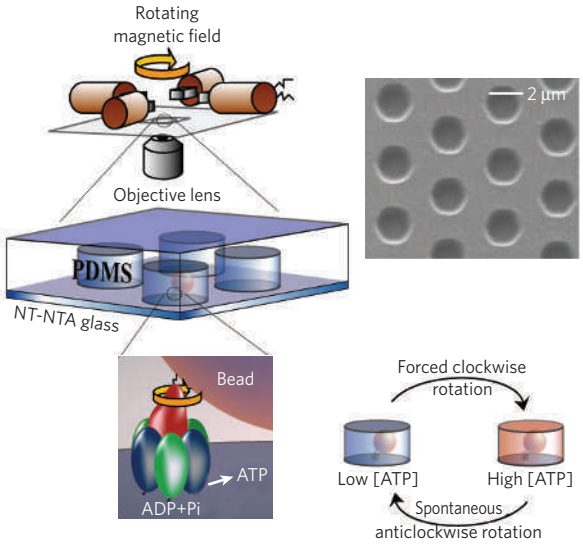

Figure 3 | Schematic of F_1ATPase rotary motor enzyme to which a magnetic bead is attached. The bead can be used to drive or observe the motion of the motor. Each enzyme is isolated in a reaction chamber of radius ~1 μm (shown schematically and in an electron micrograph) that contains the reaction products[47]. (Image courtesy of H. Noji, University of Tokyo, Japan.)

neurons cultured on the electrode arrays. The Fromhertz group has used arrays of electrodes connected to on-chip arrays of transistors to amplify voltage signals near their source in order to improve the signal-to-noise ratio[42]. With the provision of active electronics to the chip, additional signal processing and logic functions could be incorporated into the device.

In addition to the integration of electrical function with optical analysis, the chip-oriented approach can be coupled with biochemical patterning to interface more effectively to biological systems. For example, James *et al.* used methods for patterning proteins in registry with electrode arrays[43,44]. The patterned proteins guided the development of neuronal-cell growth on the electrode surface that was used to record signals from selected points in an organized cell culture. The ability to use patterned cell-growth factors, chemotactic agents or selective binding factors, such as antibodies, provides a method for interfacing the powerful analytical capabilities engineered into the inorganic devices with organized cellular systems.

As noted in the Review on cell biology in this issue (page 403), patterning of active biomaterials on surfaces can be used for cell-based lab-on-a chip assays. However, patterning at subcellular dimensions makes it possible to orient cells with respect to chip elements. The patterns themselves can be used to investigate cell responses to localized stimuli and to observe molecular-scale responses. For example, Orth *et al.* used a high-resolution method of patterning to create patterned lipid bilayers containing antigens to mammalian immune-system cells[45]. The cell-surface receptors clustered in the same pattern and resulted in cell activation. Wu *et al.* used this approach to investigate targeting in transmembrane signalling events[46]. Using fluorescence microscopy with exogeneous or genetically encoded fluorescent probes, the stimulated redistribution membrane, cytoskeletal and cytoplasmic components were tracked (Fig. 2). This allowed the testing of hypotheses about the formation of so-called lipid rafts, and demonstrated decoupling of the inner and outer leaflets of the cell membrane. Similar to the previous examples, the ability to create large numbers of essentially identical chemical stimuli allowed the system to be replicated and presented to numerous cells. The use of the patterned biochemical chip in conjunction with fluorescence microscopy provided an unambiguous visualization of the cellular response to defined stimuli and the capability to quantify it.

Planar devices for single-molecule observation

Simple chips with nanoscale features can be used as windows onto the activity of single molecules, extending the capability for observation of life processes to the molecular scale. The Noji group created an array of individual femtolitre chemical chambers on a chip for studies of the activity of individual F_1ATPase enzyme molecules bound to a surface[47,48]. In this work, the group formed small, optically transparent polymeric chambers to isolate the chemistry associated with an individual enzyme (Fig. 3). The ATPase enzyme is a rotary motor, and by attaching a magnetic bead to the rotating part of the molecule, the rotation of the molecule could be observed optically. The torque on the magnetic bead could be controlled by application of a rotating magnetic field. The researchers were able to count the rotations of the motor and calculate the efficiency as the enzyme motor consumed its ATP fuel. Because the volume of solution containing ATP was very small, the individual enzyme's activity in depleting the available energy source could be observed in reasonable experimental timescales. In a demonstration of incredible control, the individual motors could be driven in the opposite direction by the rotating magnetic field to synthesize countable ATP molecules.

This experiment provides unique insights into the activity of functioning biomolecules, directly addressing questions of kinetics, rates and efficiencies. This is an example of a potentially revolutionary class of chip-based devices in which the observation and control of large numbers of individual molecules are used as detectors of identifiable individual chemical events or components of a chemical synthesis system.

Active enzymes can be optically isolated for measurement by techniques that allow the observation of individual reaction events at high concentrations. The previously discussed approach used physical confinement of reactants to limit the reaction to a small volume. With optical confinement to a small volume surrounding an active enzyme, the observation of optically stimulated or interrogated processes can be localized to observe chemical activity specific to the isolated enzyme. Levene *et al.* described an array of subwavelength-diameter metallic apertures (Fig. 4) that confine light to dimensions well below the diffraction limit in three dimensions[49]. Light incident on this metallic structure, which can be considered as a metallic waveguide operated at a frequency below the cutoff frequency, is rapidly attenuated in the direction of propagation. Because it has no propagating waveguide modes, it is termed a zero-mode waveguide. With a diameter of a few tens of nanometres, this attenuation length becomes less than the film thickness. The transverse dimensions of the light are, of course, limited by the dimensions of the aperture. Other means of optical confinement include the use of metallic tips to concentrate optical excitation[50].

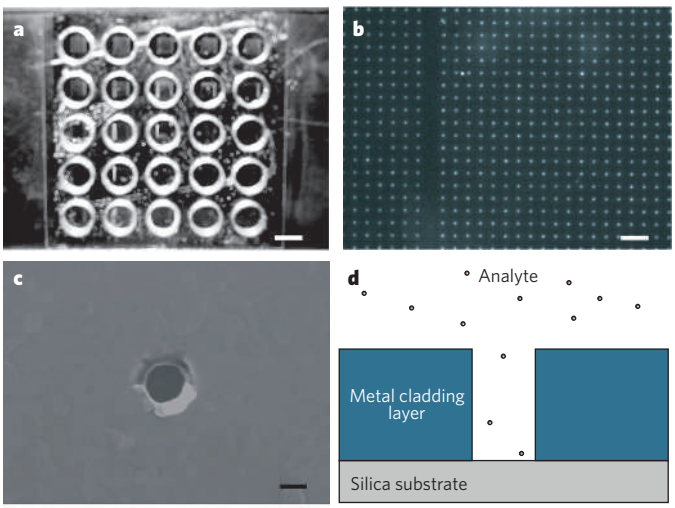

Figure 4 | Arrays of metallic apertures used for optical observation of individual molecules. Array of polymer wells containing arrays of zero-mode waveguides (**a**) shown in electron micrograph (**b**) and in higher magnification (**c**). Also shown (**d**) is a schematic cross-section of the metallic aperture with analyte solution on the metal side[49].

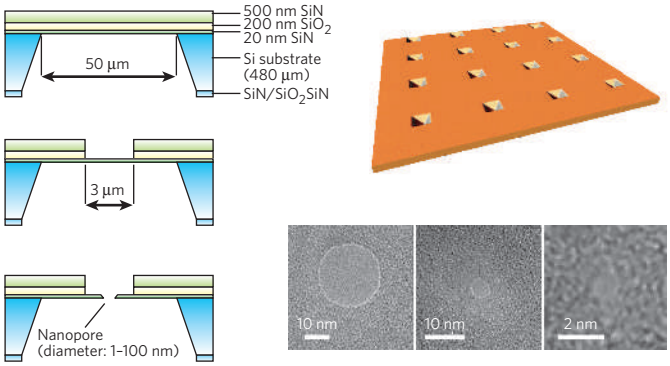

Figure 5 | Nanopores used for DNA translocation studies. Left: cross-sectional schematic of an engineered nanopore in silicon nitride showing three phases of the pore fabrication. Top right: AFM image of an array of engineered pores formed on a chip. Bottom right: transmission electron micrograph of three different pores. (Images courtesy of C. Dekker[83], University of Delft, The Netherlands, and the American Chemical Society.)

The net effect is an optical excitation volume of the order of zeptolitres (10^{-21}), significantly smaller than could, for example, be obtained by total internal reflection illumination, which can confine light to the evanescent field in one direction only. If the light is used to stimulate fluorescence in molecules in a solution covering the zero-mode waveguide at a concentration, C, the average number of molecules, N, in the optical excitation volume, V, is $N=CV$. With an excitation volume of 10^{-21} litres, $N=1$ at a concentration of 10^{21} litres^{-1}, or 1.7 mM. The behaviour of a single optically labelled entity can therefore be observed at high concentrations. If the fluorescent molecule is freely diffusing in the liquid, then the residence time in the optical excitation volume is determined by the diffusion coefficient of the molecule. By observing this time for individual molecules, the concentration of entities with different diffusion coefficients can be determined. This can be used, for example, to measure the degree of oligomerization of a biopolymer[51]. The differences in temporal fluorescent behaviour are even more pronounced in cases in which a species is permanently bound, as has been observed for the enzymatic synthesis of double-stranded DNA by DNA polymerase[49]. In this case, non-bound fluorescent species are only transiently fluorescent during their diffusive motion through the excitation region. Bound species are fluorescent for times limited by fluorescent bleaching or other processes for termination of the fluorescence. With an array, a large number of independent observation volumes can be used for the observation of enzymatic or other optically distinguishable chemical events[49–53].

Devices consisting of nanoscale pores in an electrically insulating layer are being pursued as probes of molecular structure[54–59]. In these devices, ion current passing through the narrow pore is modulated, as it passes through the pore, by the presence or nature of a large biopolymer molecule such as DNA. Naturally occurring membrane proteins such as α-haemolysin have well-defined pore dimensions, of the order of a nanometre. Engineered pores, fabricated by various forms of etching, can form similar apertures in an insulating membrane. Figure 5 shows a schematic and electron micrograph, from the Dekker group, showing apertures a few nanometres in diameter formed in a silicon nitride membrane[56]. When DNA in solution is electrically driven through such a pore, the presence of the DNA influences the passage of other mobile ions in solution through the narrow pore, and the transit of the DNA can be detected by changes in the current measured through the pore. In the simplest case, if the electrically driven speed of the molecule is known, the length of an extended DNA molecule can be inferred from the transit time. Confirmation of the molecule is observed as discrete differences in the current levels corresponding to the condition of a single strand — or a double, or triple, and so on — of a folded DNA molecule passing through the pore. Because of the strong motivation for rapid genetic sequencing or analysis, efforts are under way to extract the identity of DNA bases by resolvable electrical differences as they pass through the pore, but the resolution requirements, signal-to-noise ratio in the current signal, and the subtle differences in electrical signatures of different bases make this a difficult task. The nanopores, however, are simple but powerful biophysical probes of molecular confirmation, and a means of observing and controlling the forces on biopolymers in fluid.

Single-molecule optical analysis in flowing systems

Optical techniques are being developed for observing, detecting, analysing and quantifying single molecules. Labelling a molecule of interest with a bright fluorescent dye molecule or luminescent semiconductor particle allows selected molecules to be located and tracked by optical microscopy. Combining chemically-specific binding with the label makes it possible to identify a specific molecular species. The general approach of selective fluorescent labelling is widely used in imaging and immunoassays, and, as a result, labelling chemistry is well developed. Adapting the fluorescent approaches to analysis requires integration with fluidics and dealing with the noise limits inherent in single-molecule detection. In addition to the chemical identification imparted by specific binding chemistry, single-molecule methods can also be used to identify other features of a molecule, such as the diffusion rate, electrophoretic mobility, or rate of passage through a mechanical constriction or pore. In appropriate systems, the single-molecule approach can provide unique possibilities in quantification and dealing with limited samples, such as one from a single cell. Further to identification of chemical species or character, a significant application of single-molecule analysis is geared towards extracting genetic information from nucleic acids. A set of techniques is being developed for analysing individual molecules, with the goal of more rapid genetic or RNA expression analysis.

Optical approaches allow molecules of interest to be highlighted by labelling, but to make single-molecule approaches practical this must be coupled to microfluidic systems to efficiently deliver molecules to the optical analytical system[60–70]. Confocal optical approaches provide a direct method with which to excite and detect fluorescence from a microfluidic system. A basic system is shown in the schematic of Fig. 6. Current research approaches tend to use large-scale microscopes, lasers

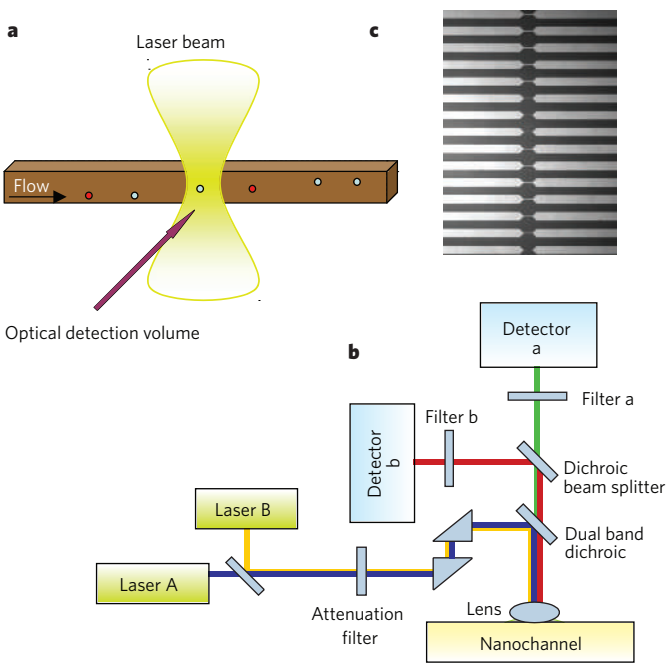

Figure 6 | Fluidic channel system for single-molecule optical measurements. **a**, Schematic of a fluid channel with optical excitation and detection volume. **b**, Schematic of optical set up. **c**, Image of 15 parallel fluid channels (pale grey) with constricted analysis region (centre)[61].

and detectors because of the availability of these systems. It should be clear, however, that the optical systems could also be miniaturized into more integrated and functional chips.

One unique single-molecule approach is to count individual molecules as they pass an interrogation region. With spectrally identifiable labels, the exact concentrations of different species could be obtained by directly counting the numbers of each molecular species. As signal-to-noise ratio is important, it is desirable to limit the size of the excitation volume to reduce background from scattering or intrinsic fluorescence of unlabelled species in the excitation volume. This motivates the use of confinement of the excitation by limiting either the optical illumination size or the physical size of the channel. Decreasing the optical excitation below the channel width reduces the detection efficiency and makes it possibile that not all molecules in the solution will be detected. Reducing the physical size of the channel improves the optical signal-to-noise ratio, and increases the interaction with the channel surfaces. The optimum in the situation is dictated by the nature of the analysis to be performed, including the concentration range of the molecules, the volume of sample and the strength of surface interactions. It seems likely that devices will be designed for specific applications.

Single-molecule approaches make it possible to detect single binding events, in addition to simply counting differentially labelled species. For example, individual binding events can be detected by simultaneously detecting the presence of fluorescence from two differently labelled molecules. Labels can be formed by pairs of molecules in which excitation energy is transferred from the donor member of the pair to the acceptor of fluorescent excitation in a fluorescence-resonance-energy-transfer or Förster-resonance-energy-transfer (FRET) process. In this case, the emission will be observed only when the binding takes place, bringing donor and acceptor into close proximity. In a microfluidic system in which the molecule of interest has a charge and an electric field is used to drive the molecule, a measure of the electrophoretic mobility can be determined from the transit time through a channel of known length[64]. Changes in mobility could indicate the difference between an unbound labelled capture molecule and a labelled capture molecule bound to a protein. The combination of microfluidics and electrically or pressure driven fluid systems creates integrated design possibilities. Parallelism from many fluid and optical channels could be designed with modern optoelectronic technology. Planar waveguide excitation, out of plane diffractive optics, laser arrays and detector arrays are all readily available to be adapted for miniaturized systems.

In addition to counting and determining the chemical identity of molecules from specific binding chemistry, the conformation and physical properties of individual molecules can be investigated in nanofluidic systems[71–80]. This can be considered to be the chip-based analogue of the probe and optical-tweezer techniques discussed above. A number of studies have been done on the sorting of DNA molecules by size in nanostructures. When associated with restriction digests, the sizes of the DNA fragments contain information on the sequence information in the original longer DNA molecules. Rapid measurement of DNA fragment size was demonstrated in a series of nanostructures with dimension smaller than the radius of gyration of the DNA molecule in free solution. In the so-called entropic traps[75,78], the rate at which a molecule could enter the constriction was length-dependent, and this dependence was used to separate bands of DNA in electrophoretically driven microfluidic channels with nanoscale constrictions. The separation was much more rapid than that achievable with conventional gel-based techniques.

Similar entropic forces in nanochannels have been used to control forces on individual molecules and to elongate them for study[71–80]. When DNA is driven into a fluid channel with transverse dimensions much less than the radius of gyration, or, even more critically, when the dimensions become comparable to the persistence length of the polymer, the confinement exerts forces on the molecule that push it from the constrained region[73] (Fig. 7). The effect of the entropic forces can be used to measure effectively the length of the molecule by the rate of passage into the constrained region[76]. Control of the molecular confirmation also allows analysis of DNA in the context of the overall chromosome.

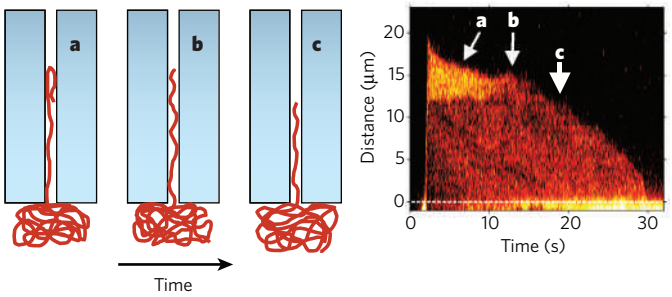

Figure 7 | Schematic and images of DNA retraction from a nanochannel. Diagram (left) showing three different time stages (**a**–**c**) of the entropically driven motion of a single DNA molecule at the interface between a microchannel and a nanochannel. After being electrophoretically driven into the channel as a 'hair pin' loop, the molecule unfolds as it is entropically pulled from the nanochannel by forces acting only on the long end. The image on the right shows stacked fluorescence images of a molecule as a function of time, demonstrating the recoiling and straightening process. The looped (**a**) and then the straightened (**b**, **c**) condition of the molecule are indicated. Once straightened, the molecule can be manipulated and positioned in the channel as an unfolded linear molecule[73].

Towards this end, Austin's group demonstrated the activity of restriction enzymes, cutting DNA at prescribed sequence locations, in an extended single molecule in a nanochannel[77].

Because of interest in their underlying sequence, DNA and RNA represent important targets for single-molecule studies, but the same concepts and physical methods of characterizing biomolecules are being extended to proteins and other compounds. In cases in which chemical differences accompany the physical differences, the situation is more complex, complicating the interpretation of phenomena for analysis. The approach for analysis, however, combining microfluidics, nanoscale structures and optical detection, creates a range of opportunities for molecular analysis.

Outlook for highly functional integrated systems

The current types of research-chip device comprise fairly simple structures, as we have seen above, such as two-dimensional arrays of apertures or reaction chambers to isolate individual molecules for study. In these simple initial devices the advantages of massively parallel replication of identical units with 10^5, 10^6 or more individual reaction or observation areas can be seen. In fluid channel systems that permit one-dimensional arrays, the degree of parallelism could easily be ~10^3. It is clear that the scale and levels of integration are compatible with active optical and electronic devices in today's integrated electronic and optoelectronic systems, and carrying out millions of parallel optical or electronic measurements is a realistic possibility. Because optical beams can traverse free space and fluid systems can be made transparent, the integration with external optical systems is possibly the most straightforward initial direction of integration. Electrical techniques require direct contact with the analyte and generally also require wires for interconnects, which creates more challenges for integration. The advances in this area are likely to be important for determining the breadth of utility of such lab-chip devices.

Opportunities for single-molecule analysis include the ability to characterize small volumes of complex mixtures such as one would obtain from a single cell. The ability to characterize the differences in populations of cells could be greatly advanced by lab-on-a-chip approaches that could rapidly quantify the levels of a set of proteins of interest in a selected cell at a particular time. As previously noted, the ability to handle cells and present controlled stimuli to cells is developing along with techniques that could help to analyse the function of these cells. In a similar way, rapid detection of the expression of RNA or the genetic make-up of individual cells could be accessed by such analytical systems. The capacity for sensitive and rapid analysis of the complex chemical content of body fluids could enable new medical diagnostic approaches, identifying the markers for disease at a stage at which treatment can be most effective.

The goal of substantially more rapid and inexpensive sequencing of entire genomes is now well established. The '$1,000 genome' is now a stated target of the US National Institutes of Health[81]. A range of approaches to this goal is being considered, including single-molecule approaches. The combination of greater understanding of biopolymer physics and the ability to integrate fluidics is presenting new opportunities and research directions.

A valuable device would allow rapid, less invasive analysis of complex biological fluids for medical diagnosis and monitoring of therapies. A range of technologies is being directed towards this general goal. The tool box of lab-on-a-chip methods, and particularly single-molecule approaches, may, in the long term, enable the engineering of a number of highly functional devices for specific diagnostic targets.

Biological and medical applications are viewed as areas of high potential impact of single-molecule approaches. Perhaps a biological analogy could provide insights into how engineered systems with controllable enzymes could be used to 'manufacture' small amounts of a large number of compounds in combinatorial approaches. Large libraries of such synthesized compounds could then be studied by comparably miniaturized systems. In any case, the existence of natural biological systems that have the ability to sense remarkably small numbers of molecules in chemically noisy environments provides guidance on how to best engineer artificial systems to operate most effectively in environments in which the statistics of signalling with small numbers of molecules[82] and time limits associated with molecular motion are the fundamental limits of operation. Living cells have evolved clearly viable approaches to sensing and reacting to their environment on the basis of the analysis of single-molecule events. ■

1. Manz, A. *et al.* Miniaturized total chemical analysis systems — a novel concept for chemical sensing. *Sens. Actuators B* **1,** 244–248 (1990).
2. Muller, D. J., Amrein, M. & Engle, A. Adsorption of biological molecules to a solid support for scanning probe microscopy. *J. Struct. Biol.* **119,** 172–188 (1997).
3. Stipe, B. C. A variable temperature scanning tunneling microscope capable of single-molecule vibrational spectroscopy. *Rev. Sci. Inst.* **70,** 133–136 (1999).
4. Hansma, H. & Hoch, J. Biomolecular imaging with the atomic force microscope. *Annu. Rev. Biophys. Biomol. Struct.* **23,** 115–134 (1994).
5. Bustamante, C. & Keller, D. Scanning force microscopy in biology. *Phys. Today* **48,** 32–38 (1995).
6. Reif, M. *et al.* Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science* **275,** 1295–1297 (1997).
7. Reif, M. *et al.* Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* **276,** 1109–1112 (1997).
8. Carrion-Vazquez, M. *et al.* Mechanical and chemical unfolding of a single protein: a comparison. *Proc. Natl Acad Sci. USA* **96,** 3694–3699 (1999).
9. Fisher, T. E. Stretching single molecules into novel confirmations using the atomic force microscope. *Nature Struct. Biol.* **7,** 719–724 (2000).
10. Ashkin, A. *et al.* Observation of a single-beam gradient force optical trap for dielectric particles. *Opt. Lett.* **11,** 288–290 (1986).
11. Ashkin, A. *et al.* Optical trapping and manipulation of single cells using infrared laser beams. *Nature* **330,** 769–771 (1987).
12. Wang, M. D. *et al.* Stretching DNA with optical tweezers. *Biophys. J.* **72,** 1335–1346 (1997).
13. Grier, D. G. A revolution in optical manipulation. *Nature* **424,** 810–816 (2003).
14. Sischka, A. *et al.* Compact microscope-based optical tweezers system for molecular manipulations. *Rev. Sci. Inst.* **74,** 4827–4831 (2003).
15. Mehta, A. *et al.* Single-molecule biomechanics with optical methods. *Science* **283,** 1689–1695 (1999).
16. Neher E. & Sakmann, B. Single-channel currents recorded for membrane of denervated frog muscle fibres. *Nature* **260,** 799–802 (1976).
17. Mijatovic, D., Eijkel, J. C. & van den Berg, A. Technologies for nanofluidic systems: top-down vs. bottom-up — a review. *Lab Chip* **5,** 492–500 (2005).
18. Craighead, H. G. Nanoelectromechanical systems. *Science* **290,** 1532 (2000).
19. Beebe, D. J., Mensing, G. A. & Walker, G. M. Physics and applications of microfluidics in biology. *Annu. Rev. Biomed. Eng.* **4,** 261–286 (2002).
20. Quake, S. R. & Scherer, A. From micro- to nanofabrication with soft materials. *Science* **290,** 1536–1540 (2000).
21. Schwarz, M. A. & Hauser, P. C. Recent developments in detection methods for microfabricated analytical devices. *Lab Chip* **1,** 1–6 (2001).
22. Liu, J., Hansen, C. & Quake, S. R. Solving the "world to chip" interface problem with a microfluidic matrix. *Anal. Chem.* **75,** 4718–4723 (2003).
23. Ng, J. M. K., Gitlin, I., Stroock, A. D. & Whitesides, G. M. Components for integrated poly(dimethylsiloxane) microfluidic systems. *Electrophoresis* **23,** 3461–3473 (2002).
24. Zheng, G. *et al.* Multiplexed electrical detection of cancer markers with nanowire sensor arrays. *Nature Biotechnol.* **23,** 1294–1301 (2005).
25. Patolsky, F. & Lieber, C. M. Nanowire nanosensors. *Mater. Today* **8,** 20–28 (2005).
26. Rosenblatt, S. High performance electrolyte-gated carbon nanotube transistors. *Nano Lett.* **2,** 869–972 (2002).
27. Sazonova, V. *et al.* A tunable carbon nanotube electromechancial oscillator. *Nature* **431,** 284–287 (2005).
28. Burg, T. & Manalis, S. Suspended microchannel resonators for biomolecular detection. *Appl. Phys. Lett.* **83,** 2698–2701 (2003).
29. Verbridge, S. *et al.* Suspended glass nanochannels coupled with microstructures for single molecule detection. *J. Appl. Phys.* **97,** 124317–124320 (2005).
30. Ilic, B. *et al.* Enumeration of DNA molecules bound to a nanomechanical oscillator. *Nano Lett.* **5,** 925–929 (2005).
31. Song, S., Singh, A. K. & Kirby, B. J. Electrophoretic concentration of proteins at laser-patterned porous membranes. *Anal. Chem.* **76,** 4589–4592 (2004).
32. Schmidt, C., Mayer, M. & Vogel, H. A chip-based biosensor for the functional analysis of single ion channels. *Angew. Chem. Int. Ed.* **39,** 3137–3140 (2000).
33. Suzuki, H. *et al.* Highly reproducible method of planar lipid bilayer reconstitution in polymethyl methacrylate microfluidic chip. *Langmuir* **22,** 1937–1942 (2006).
34. Fertig, N. *et al.* Microstructured glass chip for ion-channel electrophysiology. *Phys. Rev. E. Stat. Nonlin. Soft Matter Phys.* **64,** 040901-1–040901-4 (2001).
35. Fertig, N., Blick, R. H. & Behrends, J. C. Whole cell patch clamp recording performed on a planar glass chip: electrophysiology on a chip. *Biophys. J.* **82,** 3056–3062 (2002).
36. Klemic, K. B. *et al.* Micromolded PDMS planar electrode allows patch-clamp electrical recordings from cells. *Biosens. Bioelectron.* **17,** 597–604 (2002).
37. Groves, J. T., Ulman, N. & Boxer, S. G. Micropatterning fluid bilayers on solid supports. *Science* **275,** 651–653 (1997).
38. Pantoja, R. *et al.* Bilayer reconsistution of voltage-dependent ion channels using a microfabricated silicon chip. *Biophys. J.* **81,** 2389–2394 (2001).
39. Xu, L. *et al.* Ion-channel assay technologies: quo vadis? *Drug Discov. Today* **6,** 1278–1287 (2001).
40. Dias, A. *et al.* An electrochemical detector array to study cell biology on the nanoscale. *Nanotechnology* **13,** 285–289 (2002).
41. Hafez, I. *et al.* Electrochemical imaging of fusion pore openings by electrochemical detector arrays. *Proc. Natl Acad. Sci. USA* **102,** 13879–13884 (2005).
42. Zech, G. & Fromhertz, P. Noninvasive neuroelectronic interfacing wth synaptically connected snail neurons immobilized on a semiconductor chip. *Proc. Natl Acad. Sci. USA* **98,** 10457–10462 (2001).
43. James, C. D. *et al.* Extracellular recordings from patterned neuronal networks using planar microelectrode arrays. *IEEE Trans. Biomed. Eng.* **51,** 1640–1648 (2004).
44. Oliva, A. A. *et al.* Patterning axonal guidance molecules using a novel strategy for microcontact printing. *Neurochem. Res.* **28,** 1639–1648 (2003).
45. Orth, R. N. *et al.* Mast cell activiation on patterned lipid bilayers of subcellular dimensions. *Langmuir* **19,** 1599–1605 (2003).
46. Wu, M. *et al.* Visualization of plasma membrane compartmentalization with patterned lipid bilayers. *Proc. Natl Acad. Sci. USA* **101,** 13798–13803 (2004).
47. Rondelez, Y. *et al.* Highly coupled ATP synthesis by F_1-ATPase single molecules. *Nature* **433,** 773–777 (2005).
48. Rondelez, Y. *et al.* Microfabricated arrays of femtoliter chambers allow single molecule enzymology. *Nature Biotechnol.* **23,** 361–365 (2005).
49. Levene, M. J. *et al.* Zero-mode waveguides for single-molecule analysis at high concentrations. *Science* **299,** 682–686 (2003).
50. Gerton, J. M., Wade, L. A., Lessard, G. A., Ma, Z. & Quake, S. R. Tip-enhanced fluorescence microscopy at 10 nanometer resolution. *Phys. Rev. Lett.* **93,** 180801 (2004).
51. Samiee, K. T. *et al.* λ-Repressor oligomerization kinetics at high concentrations using fluorescence correlation spectroscopy in zero-mode waveguides. *Biophys. J.* **88,** 2145–2153 (2005).
52. Samiee, K. *et al.* Zero mode waveguides for single molecule spectroscopy on lipid membranes. *Biophys. J.* **90,** 3288–3299 (2006).
53. Rigneault, H. J. *et al.* Enhancement of single molecule fluorescence detection in subwavelength apertures. *Phys. Rev. Lett.* **95,** 117401–117404 (2005).
54. Kasianowicz, J. *et al.* Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl Acad. Sci. USA* **93,** 13770–13773 (1996).
55. Deamer, D. W. & Branton, D. Characterization of nucleic acids by nanopore analysis. *Acc. Chem. Res.* **35,** 817–825 (2002).
56. Storm, A. J. *et al.* Fast DNA translocation through a solid-state nanopore. *Nano Lett.* **5,** 1193–1197 (2005).
57. Heng, J. B. *et al.* Sizing DNA using a nanometer-diameter pore. *Biophys. J.* **87,** 2905–2911 (2004).
58. Li, J. *et al.* DNA molecules and configurations in a solid state nanopore microscope. *Nature Mater.* **2,** 611–615 (2003).
59. Mathe, J. *et al.* Nanopore unzipping of individual DNA hairpin molecules. *Biophys. J.* **87,** 3205–3212 (2004).
60. Goodwin, P. M., Nolan, R. L. & Cai, H. Single-molecule spectroscopy for nucleic acid analysis: a new approach for disease detection and genomic analysis. *Curr. Pharm. Biotechnol.* **5,** 271–278 (2004).
61. Stavis, S. M. Single molecule studies of quantum dot conjugates in a submicrometer fluidic channel. *Lab Chip* **5,** 337–343 (2005).
62. Stavis, S. M. Detection and identification of nucleic acid engineered fluorescent labels in submicrometre fluidic channels. *Nanotechnology* **16,** S314–S323 (2005).
63. Foquet, M. DNA fragment sizing by single-molecule detection in submicrometer-sized closed fluidic channels. *Anal. Chem.* **74,** 1415–1422 (2002).
64. Stavis, S. M. Single-molecule mobility and spectral measurements in submicrometer fluidic channels. *J. Appl. Phys.* **98,** 044903-1–044903-5 (2005).
65. Campbell, L. C. *et al.* Electrophoretic manipulation of single DNA molecules in nanofabricated capillaries. *Lab Chip* **4,** 225–229 (2004).
66. Mogensen, K. B., El-Ali, J., Wolff, A. & Kutter, J. P. Integration of polymer waveguides for optical detection in microfabricated chemical analysis systems. *Appl. Opt.* **42,** 4072–4079 (2003).
67. Foquet, M. E. *et al.* Fabrication of microcapillaries and waveguides for single molecule detection. *Proc. SPIE* **3258,** 141–147 (1998).
68. Dittrich, P. S. & Manz, A. Single-molecule fluorescence detection in microfluidic channels — the Holy Grail in μTAS? *Anal. Bioanal. Chem.* **382,** 1771–1782 (2005).

69. Tegenfeldt, J. *et al.* Micro- and nanofluidics for DNA analysis. *Anal. Bioanal. Chem.* **378,** 1678–1692 (2004).
70. Kricka, L. Revolution on a square centimeter. *Nature Biotechnol.* **16,** 513–514 (1998).
71. Tegenfeldt, J. O. *et al.* Stretching DNA in nanochannels. *Biophys. J.* **86,** 596A (2004).
72. Tegenfeldt, J. O. *et al.* The dynamics of genomic-length DNA molecules in 100-nm channels. *Proc. Natl Acad. Sci. USA* **101,** 10979–10983 (2004).
73. Mannion, J. *et al.* Conformational analysis of single DNA molecules undergoing entropically induced motion in nanochannels. *Biophys. J.* **90,** 4538–4545 (2006).
74. Kung, C.-Y. *et al.* Confinement and manipulation of individual molecules in attoliter volumes. *Anal. Chem.* **70,** 658–661 (1998).
75. Han, J. *et al.* Entropic trapping and escape of long DNA molecules at submicron constrictions. *Phys. Rev. Lett.* **83,** 1688–1691 (1999).
76. Cabodi, M. *et al.* Entropic recoil separation of long DNA molecules. *Anal. Chem.* **74,** 5169–5174 (2002).
77. Riehn, R. *et al.* Restriction mapping in nanofluidic devices. *Proc. Natl Acad. Sci. USA* **102,** 10012–10016 (2005).
78. Han, J. & Craighead, H. G. Separation of long DNA molecules in a microfabricated entropic trap array. *Science* **288,** 1026–1029 (2000).
79. Perkins, T. T., Smith, D. E., Larson, R. G. & Chu, S. Stretching of a single tethered polymer in a uniform flow. *Science* **268,** 83–87 (1994).
80. Bakajin, O. B. *et al.* Electrodynamic stretching of DNA in confined environments. *Phys. Rev. Lett.* **80,** 2737–2740 (1998).
81. Service, R. The race for $1000 genome. *Science* **311,** 1544–1546 (2006).
82. Bialek, W. & Setayeshgar, S. Physical limits to biochemical signaling. *Proc. Natl Acad. Sci. USA* **102,** 10040–10045 (2005).
83. Krapf, D. *et al.* Fabrication and characterization of nanopore-based electrodes with radii down to 2 nm. *Nano Lett.* **6,** 105–109 (2006).

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