INNOVATION

Life-on-a-chip

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Mechanistic studies of cellular processes are usually carried out with large populations of cells. However, parameters that are measured as averages of large populations can be misleading. For instance, an apparently linear response to a signal could, in fact, reflect an increasing number of cells in the population that have switched from 'off' to 'on', rather than a graded increase in response by all the cells. At present, the study of single cells is challenging, but new technologies mean it might soon be a reality.

The increasing availability of genome sequences for both prokaryotes and eukaryotes is laying the foundation for a revolution that will ultimately transform biology from a largely descriptive and reductionist discipline into a fundamentally predictive science. The growing ability to analyse whole biological systems on the basis of genomic information is creating snapshots of cells at the transcriptional and translational level, which are providing preliminary insights into cellular complexity. However, to understand complex molecular outcomes it will be necessary to determine how individual parts are integrated in time and space to form complex, dynamic cellular functions, and how cellular interactions create higher-order functions. Such analyses require the simultaneous measurement of multiple variables in living cells in real time. Traditionally, studies of complex processes in cells have been carried out with large populations of cells by the 'blender' approach — by measuring parameters in either whole cells or cell extracts, and reporting an averaged value. However, it has long been known that many processes result in cellular heterogeneity, apparently as a result of STOCHASTICITY in biological mechanisms — the random element of both timing and molecular partitioning[1–4]. Examples of heterogeneous phenotypes in *Escherichia coli* include phase variation in type I pili[5–8], lambda phage infection[9,10] and control of both the lactose and arabinose promoters[11,12]. In the latter two cases the systems are binary, that is, they are either 'on' or 'off'. In such cases, populations of cells exposed to subsaturating inducer concentrations are mixtures of some cells that are on and other cells that are off. Therefore, intermediate values of expression that are obtained in bulk cultures reflect changes in the numbers of cells in each subpopulation in the on configuration, rather than a graded response in each cell (FIG. 1). Such heterogeneity is widespread in bacteria, and evidence indicates that it is also common in eukaryotic cells[3,4,13], in which heterogeneity regarding the activation of signalling pathways might lead to disease states[14]. A second problem with functional genomic approaches in bulk cultures is the concentration step. It is common to concentrate samples by subjecting the cells to filtration or centrifugation, but because cell pools, physiology and gene expression respond in timescales of seconds or minutes, the results obtained could be a measurement of a response to the concentration process rather than of a response to the conditions under test. Finally, an added benefit of single-cell studies is the requirement for less cell material, enabling studies of previously unculturable strains and rare cells. As we move into the era of functional genomics, in light of known phenotypic heterogeneity and the need to analyse cells during, rather than after a response, it is becoming imperative that functional genomics analyses are carried out at the level of individual cells, and in sufficient numbers to develop statistically meaningful data sets with regard to biological noise and specific cellular mechanisms. This should include the same kinds of global and multi-parameter studies that are, at present, being applied to bulk populations, including genetic, physiological and biochemical measurements.

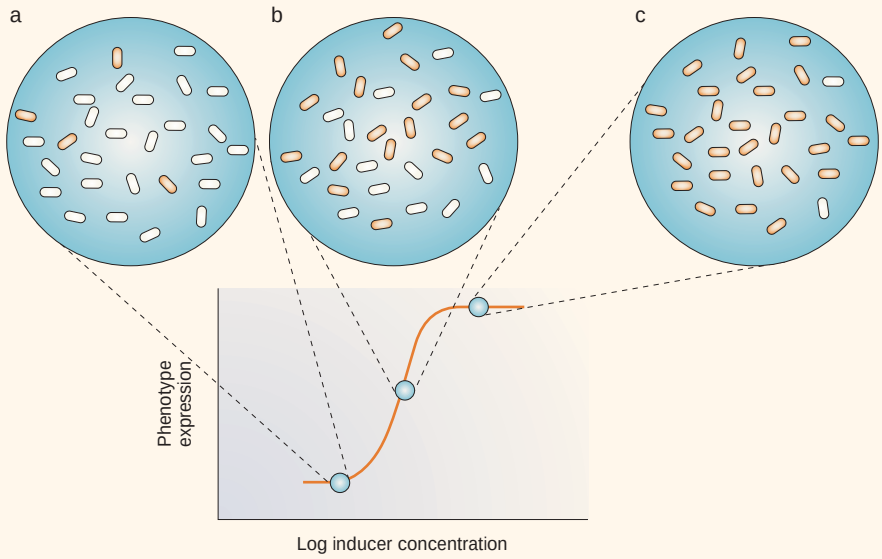

Figure 1 | **Heterogeneity in cellular response systems.** Some mechanisms that control cellular responses have been shown to be analogous to on/off switches. That is, below a threshold concentration of the signal molecule, the response is off, and above that threshold the response occurs so rapidly that it is essentially on. For individual cells, the absolute concentration of the signal molecule that 'trips the switch' varies, presumably depending on variables such as the number of receptor molecules per cell, the number of gene copies of the target per cell and the number of transporter molecules for the signal. In the latter case, bursts of synthesis of the transporter in a subpopulation of cells result in the ability of those cells to concentrate the inducer above the level that is crucial for induction. This state is maintained because the transporter is autocatalytic — its synthesis is induced by the inducer. Therefore, at a concentration of the signal molecule that is below the average threshold level, a small number of cells (orange) will turn on early (**a**). As the concentration increases to the threshold level, an increasing number of cells will turn on (**b**), and, finally, as the concentration increases to well above the threshold level, all cells will turn on except those that are non-responding (owing to mutation or death) (**c**). When this information is plotted for a population of cells, it seems to be a graded response to the concentration of the signal. Such results imply that in the population each cell has exhibited an intermediate response. In fact, for this type of mechanism, some cells have no response whereas others have a full response. The incorrect interpretation of the mechanism leads to incorrect predictions of cellular responses. For instance, in order to manipulate the response of an on/off switch involving an autocatalytic transporter to the inducer, it is necessary to alter the regulation of the transporter. However, for a non-autocatalytic system the target would be the regulatory protein itself.

Technology for single-cell analysis

Although population-based analyses are still common, an increasing number of studies involve measurements in individual cells. For example, multiple fluorescent reporters for proteins or small molecules can be monitored in real time in living cells using a microscope or fluorescence-activated cell sorting (FACS) analysis[15–19], reverse trancription-polymerase chain reaction (RT-PCR) has been used to analyse single-cell gene expression[20,21], techniques are available for single-cell enzyme measurements[19,22,23] and single-cell expression microarrays have been reported[13]. These types of evolving technologies are important steps in moving from population-based analysis to single-cell analysis. However, they are not yet ideal. They are largely confined to a single measurement technique and they are often destructive or invasive to the cell, and therefore cannot be carried out in real time to follow dynamic processes in undisturbed cells. Therefore, the results often suffer from the same difficulties as population studies, because the data might reflect cellular responses to the invasive technique rather than only to the perturbation studied. In addition, these techniques often involve expensive instrumentation that is not available to the general research laboratory. To apply such techniques broadly to biological research, significant funds for instrumentation and support staff are often required.

Integrated technology development

New technology is needed to allow the analysis of multiple parameters in individual undisturbed living cells in real time. Such technology should be capable of being multiplexed, to allow the simultaneous analysis of many cells, and it should be amenable to mass production to minimize costs. Advances in MICROFLUIDICS, microfabrication and microelectromechanical systems (MEMS) (BOX 1) (collectively known as 'lab-on-a-chip')[24–27] provide promise for platforms for such analyses, but many challenges remain in detection, cell handling and system integration. Prototypes that are capable of simple cellular analyses are now beginning to be described (BOX 2), and are proof of the microsystem-based single-cell analysis principle. In this report, we describe some of the challenges and opportunities that exist in applying integrated microsystems to single-cell analyses.

Technology needs. A core set of foundation technologies is required to manipulate and analyse individual cells. These technologies include an integrated microsystems platform for the maintenance of living cells (both eukaryotic and prokaryotic), automated microfluidic handling, trapping of cells, highly sensitive detection of small molecules and proteins, integrated controls and data-handling systems, and a cell-lysis module with downstream processing capabilities. Each of these required capabilities should be designed in a modular fashion, so that systems can be custom-assembled for each application, with individual modules capable of fastening together by direct interconnections. However, such a modular design requires careful integration of the technologies in each module, as well as integrated controls and data handling. A schematic design of such an integrated microsystem is shown in FIG. 2.

Cell handling. The core integrated microsystem platform should ideally combine very low volume fluid handling (microfluidics) with a miniaturized temperature-controlled chamber and cell-trapping system. Microsystem-based platforms for housing living cells at controlled temperature with medium flow-through have been made[28], which are essentially micro-environmental chambers on the nanolitre to microlitre volume scale. Such observation chambers also include an optical interface for light detection and/or image capture, usually either a microscope or charge-coupled device (CCD) camera. Microfluidic systems that comprise miniature channels, valves and pumps, and

Box 1 | Microelectromechanical systems

Microelectromechanical systems (MEMS) refers to integrated systems on a chip that might include mechanical elements, sensors, actuators and electronics. MEMS devices are made by techniques that are known as microfabrication technology. In general, electronics are produced using integrated circuit process techniques, and the micromechanical components are fabricated by selectively etching away parts of the silicon wafer (the chip). Depending on the complexity of the MEMS device, new structural layers can be added to form the mechanical and electromechanical devices. MEMS devices incorporate circuits that provide a decision-making capability with the ability to sense and control the environment. Mechanical, thermal, biological, chemical, optical and magnetic sensors can be used to gather information from the environment. This information is then used to direct the actuators to respond by moving, positioning, regulating, pumping and filtering to achieve a desired outcome — for instance, sorting cells in picolitre volumes[24]. As a group, MEMS devices are characterized by a broad range of functions, such as high reliability, and relatively low production costs as they can be mass produced.

Box 2 | Microfluidic device for single-cell analysis

An example of a microfluidic device for the analysis of single cells has been described by Wheeler *et al.*[28] at Stanford University and Fluidigm Corporation. This device has the ability to capture individual cells and subsequently perfuse reagents onto selected cells for multistep cell analyses. It is made using a multilayer soft lithography technology with poly(dimethysiloxane), a soft polymer that enables fabrication of near-zero dead-volume valves. Pneumatic pressure is applied to a control channel to deform a membrane, thereby closing the fluidic channel or shutting the valve. Valves are a basic element that can be combined to form pumps and mixers.

To isolate individual cells, the device incorporates a 'T-junction' and a 'dock', which is positioned at the centre of the T-junction. A flowing solution divides into two streams at the T-junction due to the laminar flow characteristics of microfluidic systems and generates a point of stagnation at the centre of the 'T'. The dock, the walls of which form a chamber, is fabricated with small drain channels at the point of stagnation to trap a cell as the flow stream divides. Once a cell is trapped, nanolitre volumes of reagents can be delivered to the cell in milliseconds from two small delivery channels next to the cell dock. One channel delivers a shield buffer and the other delivers reagents by activating pumps and valves on the device with a digitally controlled manifold of three-way pneumatic switch valves.

This microfluidic device has been used for a cell-viability assay and to measure intracellular calcium ion concentrations, $[Ca^{2+}]_i$, such as ionophore-mediated $[Ca^{2+}]_i$ flux and crystallizable fragment-γ-receptor-mediated $[Ca^{2+}]_i$ flux. Microscale dimensions of the device chamber (the T-junction has dimensions of 75×20 μm for the width and depth, respectively), and the associated nanolitre fluid volumes mean that these assays consume 10^5 times less reagent and are 10 times faster than standard macroscale systems.

This device shows that single-cell assays are now technically feasible for some simple assays. Future single-cell studies will require the ability to provide environmental control and multiple sensors for multiparameter real-time analyses.

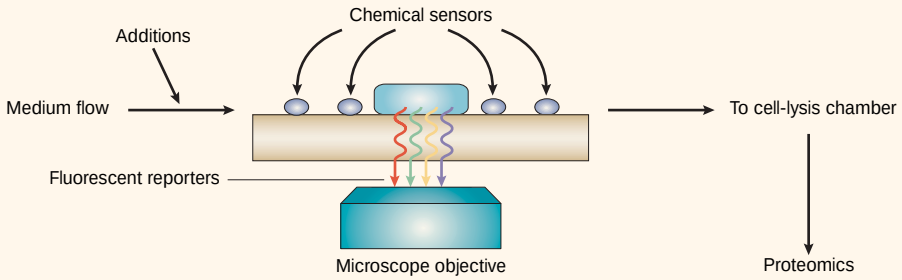

Figure 2 | **Integrated microsystem schematic.** This schematic shows the core modules in a system that is designed to analyse processes in single cells in real time.

are designed for the movement of fluids through such channels are relatively well established[25,28,29]. In addition, fabrication methods are now available to multiplex microfluidic systems to pattern a thousand functional units on a single chip[30]. Once fluids and cells enter the channel, individual cells must be sorted into observation/analysis sites and trapped there. A simple method for trapping involves the creation of a surface to which the cells will stick, such as glass or a patterned antibody layer. However, this has the disadvantage of physically tethering the cell to a surface and, in the case of the antibody tether, the time and effort that is required to generate antibodies for each biological system. Attached bacterial cells have different physiological characteristics to free-floating cells[31–33]. Similarly, in eukaryotic cells, mechanical forces that change depending on the three-dimensional state of the cells are known to affect physiological responses[34], including cytoskeleton-related physiological changes[35]. Therefore, it is desirable to develop a cell-trapping system that does not involve attachment to surfaces. Two possible options are DIELECTROPHORESIS[24] and fluidic microreactors[36]. Dielectrophoresis creates a potential well that uses alternating currents, and fluidic microreactors take advantage of eddies that form from alternating fluid flows behind a barrier. Both systems are amenable to microfabrication, and a wide variety of devices have been made for dielectrophoretic separation of particles[37,38].

Sensors. Once a cell has been moved to an analysis site and trapped there, sensors are needed to measure biologically meaningful parameters, such as pH, O_2, ions, proteins and signal molecules. Sensors are available for detecting a wide range of molecules, including those coupled to light emission and those with electrical outputs, such as microelectrodes. However, the challenge for this application is to identify sensor systems that can be miniaturized and microfabricated, amd that also have the high sensitivity that is required for detecting very small target amounts. Fluorescent intracellular probes are potentially very useful[18,19,23], and microfabricated detection systems have been reported[39]. Such systems have been applied to specific functions — for instance, Quake and colleagues have developed a microfabricated fluorescence-activated cell sorter[40]. However, fluorescent reporters are limited in their application. Many of the small-molecule probes are not taken up by prokaryotic cells, and in the case of fluorescent proteins, expression can be toxic. If the interrogation process stresses or damages the cell, it will not be possible to obtain data under conditions of non-stressed ('normal') physiology. In addition, presently available technology for simultaneous analyses is limited to a handful of emitters, greatly limiting the number of compounds that can be studied simultaneously. MICROANALYTICAL SPECTROSCOPIC METHODS[41,42] and SURFACE PLASMON RESONANCE[43] methods will be important technologies for the development of MULTIANALYTE sensor arrays for continuous monitoring of small molecules for individual cells. Both methods are potentially miniaturizable and the examples cited above have detected molecules at micromolar levels and, in at least one case, with subnanolitre sample volumes[42].

Systems integration. All of the features described above must be integrated in modules with robust interconnections. In addition, each module and its outputs must be interfaced with integrated controls and data-handling systems. It seems likely that in the near future at least, it will be desirable to have the ability to first interrogate cells in real time, then harvest and lyse them to analyse their contents. Therefore, a cell-lysis module that is connected to the observation chamber will also be important. The cell contents could then be analysed further, either by microanalytical techniques and sensor arrays, as discussed above, or by single-cell proteomics[23] and/or single-cell expression microarrays[13]. Although the feasibility of all of these techniques have been shown at the proof-of-principle stage, the next step is to miniaturize and integrate them into a single microsystem platform.

The challenge ahead is to develop the interconnections between modules and to move these prototype and/or proof-of-principle demonstrations into a set of practical, reproducible and reliable devices for use in the research laboratory. To meet this challenge will require not only innovation, but also close collaboration between the technology developers, the integration teams and the end users. A few interdisciplinary teams are forming to address specific biological questions at the single-cell level — for instance, the Cell Systems Initiative, the Nanosystems Biology Alliance and the Molecular Sciences Institute. We are involved in another team, the Microscale Life Sciences Center (MLSC),

Glossary

DIELECTROPHORESIS
The induced motion of polarizable particles in non-uniform electric fields.

ELECTROIMPEDENCE SPECTROSCOPIC METHODS
An analytical technique that supplies frequency response information for a variety of conducting materials. A signature is obtained that can be related to specific changes in conducting properties.

MICROANALYTICAL SPECTROSCOPIC METHODS
Detection methods that are based on analysis of the energies and wavelengths of radiation emitted by atoms and molecules when particular physical conditions are applied to them, and which use small (sub-microlitre) volumes and low concentrations of analyte.

MICROFLUIDICS
Fluidics in structures on micron and smaller-length scales, resulting in low turbulence, with laminar flows.

MULTIANALYTE
Multiple chemicals to be analysed.

STOCHASTICITY
Describes a phenomenon that obeys the laws of probability.

SURFACE PLASMON RESONANCE
(SPR). A phenomenon that occurs when light is reflected off thin metal films. Although incident light is totally reflected, the electromagnetic field component penetrates a short (tens of nanometres) distance into a medium of a lower refractive index, thereby creating a type of wave that is known as an exponentially detenuating evanescent wave. If the interface between the media is coated with a thin layer of metal (gold), and light is monochromatic and p-polarized, the intensity of the reflected light is reduced at a specific incident angle producing a sharp shadow, called surface plasmon resonance.

and the activities of this group will be described in more detail.

Microscale Life Sciences Center

The development of the enabling technology described above and its application to fundamental biological problems is the goal of the MLSC at the University of Washington. The MLSC is a Center of Excellence in Genomic Sciences (CEGS), funded by the National Institutes of Health (NIH) National Human Genome Research Institute (NHGRI) and was founded in August 2001. The long-term goal of the MLSC is to carry out functional genomics, a single cell at a time. The MLSC consists of several research groups with interests in technology development, microfabrication, systems integration and fundamental biology (Online FIG. 1).

Microbiological questions. Any complex biological event, such as growth, division and infection, is subject to cellular heterogeneity in populations. Such events are particularly prone to heterogeneity if they are controlled, at least in part, by autoregulated transporters that act as gates for signal molecules (FIG. 1), or by small numbers of signal or receptor molecules[1–3]. In the latter case, natural variations in the proportion of signal molecules and/or signal receptors per daughter cell can have significant effects on regulatory mechanisms when the total number of molecules is small[3,44]. For instance, if a regulatory protein is present at only 50 copies per cell, two specific daughter cells might contain 15 and 35 copies of the protein, respectively, which could significantly affect the cellular outcome. The first cell might show a low-response phenotype and the second cell might show a high-response phenotype. In the MLSC, a group of investigators is involved in studying a set of fundamental biological problems in which cellular heterogeneity is of special concern. These problems include the study of integrated metabolic networks in a bacterium, infection of eukaryotic cells by bacteria and viruses, yeast pedigree analysis and signalling by rare regulatory proteins in yeast. In each case, interpretation of mechanistic data from bulk cultures is complicated by a lack of understanding of heterogeneity. The heterogeneity might manifest itself as phenotypic subpopulations that occur in response to stimulus or stress (for instance, in metabolic or developmental heterogeneity) or subpopulations that are offset in time-dependent events (for instance, infection-related events).

Although the biological questions of interest to the MLSC seem disparate, each is united by the need for single-cell analysis. In the case of understanding integrated metabolic networks, the goal is to understand and design novel metabolic circuits in a methylotrophic bacterium[45]. Population heterogeneity interferes with this process, creating uncertainty in design targets and design goals. The aim of this project is to analyse metabolic networks in individual cells under non-stressful and stressful conditions, and during transition from one metabolic mode to another, to determine how the different parts of metabolism are coordinated and balanced. In the pedigree analysis study, yeast are used as a model to probe the relationship between ageing and cancer with chromosomal abnormalities (loss of heterozygosity) that occur at increasing frequency in yeast mother cells as they age[46]. The goal of this project is to observe events in large numbers of individual mother and daughter cells and to manipulate the daughter cells, as they are budded off so as to automate and standardize the protocol for analysing the ageing process. The second yeast study involves the use of capillary electrophoresis to separate proteins from a single cell and measure them by fluorescence[47], with the goal of detecting individual protein molecules at the single copy level, tagged with green fluorescent protein (GFP). The target will be regulatory proteins expressed at low levels, and the aim of the project is to analyse large numbers of individual cells to assess heterogeneity in the numbers of these protein molecules in each cell.

"Any complex biological event, such as growth, division and infection, is subject to cellular heterogeneity in populations."

Infection of mammalian cells by pathogenic bacteria or viruses is another broad topic for which heterogeneity is an important issue. Differences in the timing of infection events and inherent heterogeneity in both host cells and pathogenic agents make absolute synchronization of infection processes difficult. This, in turn, compromises measurements that are made on bulk populations (FIG. 3). For instance, if at early time points in the infection process, an infecting population comprises a mixture of cells that have not yet entered the infection process, cells that have already initiated infection events and cells that have progressed to a later infection stage, measurements of gene expression and physiological changes will have intermediate values rather than the true values in individual

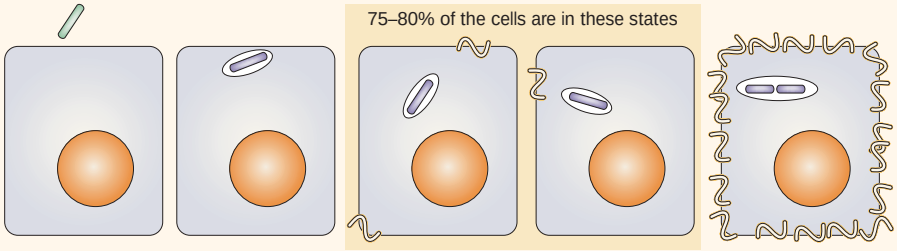

Figure 3 | **Heterogeneity in timing of infection events.** If a proportion of infected populations initiate infection at different times owing to inherent heterogeneity in both populations, the infection programme could be misinterpreted. In this fictitious example, an infecting cell enters the host cell, moves to the vacuole, then divides within the vacuole. Sample hypothetical cells are shown to illustrate the heterogeneity of an infectious population. Two parameters are measured in the infecting cell (green and purple) and one in the host cell (yellow). Green is on (relative value = 1) prior to entry to the host cell and then turns off (value of 0). Purple turns on (value of 1) after entry to the host cell. A receptor (yellow) turns on (value of 1 per receptor) at a low level after entry, and is at a very high level after division of the infecting cell in the vacuole. Inaccurate results can be obtained from bulk analyses in this early time point when the population of infecting and host cells are 75–80% synchronized. For example, one measured parameter in the infecting cell (green) could be 1 (before entry), but measured as 0.3 per infecting cell as a bulk result. A second parameter in the infecting cell (purple) could be 1 (after entry), but measured as 0.73 per infecting cell as a bulk result. Finally, a host cell parameter (yellow) could be 14 after entry, but measured as 0.3 per host cell as a bulk result. At later time points a shift in parameters will be observed, but it will be offset in timing and magnitude from the actual infection programme. In this illustrative case, on/off mechanisms of the type shown in FIG. 1 are in place, but the interpretation of bulk results would be a graded response, with a blurring of the timing and correlation to other cellular events. The key ratio of the infecting cell parameter (purple) to the host response parameter (yellow), which might be used to interpret the infection programme, would be especially misleading.

cells. Such results blur control mechanisms and timing points, and make mechanistic interpretation of outcomes problematic. Two pathogenesis systems are under study in the MLSC. In the bacterial pathogenesis system, *Salmonella enterica* serovar Typhimurium infects, and eventually kills, macrophages by invoking a unique process of proinflammatory programmed cell death that is known as pyroptosis[48]. The goal of the project is to measure events in real time during the infection processes in both the host cell and the infecting agent, and to assess the role of heterogeneity in the outcome of infection — for instance, lysis of the macrophage and replication of the bacteria. In addition, a viral pathogenesis system — HIV infection of T cells — is being studied. The goal of this work is to dissect the impact of HIV infection on gene and protein expression in naive and memory T cells. The project involves a similar set of goals to the *S. typhimurium* pathogenesis project — to work with small numbers of T cells and analyse individual infection events with respect to heterogeneity and pathogenesis outcomes.

Although each of these biological systems has a separate goal in terms of the questions addressed, commonalities in outcomes exist between them (FIG. 4). The analysis of response and heterogeneity at the single cell level, the detection of single molecules, and the dissection of complex biological outcomes, such as infection and growth, are common threads among all of the teams in the MLSC.

Integrated technology development. The MLSC is involved in the development of new technology — in particular, the application of existing technology, and the integration of both new and existing technologies into robust microsystem devices for use in addressing the biological questions described above. All of these biological questions require common, core technologies. These include the non-stressful incubation of cells, the ability to perturb cells physically and/or chemically, the monitoring of cell function (for example, respiration rates, substrate utilization and product excretion), the monitoring of gene expression and measuring the outcomes of the event (such as protein expression and metabolic changes). Initial efforts have focused on the integrated optical platform that is shown in FIG. 2, coupling microfluidic technologies and micro-environmental chamber designs with a confocal microscope as the optical analysis and image-capture system. An example microsystem design for the yeast pedigree analysis system is shown in FIG. 5.

Technology challenges for the environmental chamber include temperature control, which is being addressed with microheaters that have been shown to be effective in microsystem-embedded temperature control[49]. Cell trapping in the chamber is being accomplished both by dielectrophoresis[37,38] and fluidic microreactors[36], and proof-of-principle level modules have been built and tested for both approaches. Oxygen sensing for measuring respiration rates has been accomplished at the 10^5 cell level using an O_2-responsive porphyrin dye[50], and efforts are underway to adapt this system to small-scale measurements. Microspectroscopic[41,42] and ELECTROIMPEDENCE SPECTROSCOPIC METHODS[51] are also being developed to measure small molecules and cell characteristics.

A cell-lysis module will also be developed, so that after monitoring, the cell can be harvested and its contents rapidly assessed by capillary electrophoresis for proteomics[23], or by an as-yet-undeveloped technique involving sensor arrays for small molecules, proteins and other biologically relevant molecules.

Iterative design process. To effectively design and test such integrated lab-on-a-chip microsystems, an iterative design process has been initiated in the MLSC (FIG. 5b). In this process, the design constraints and performance targets are developed by teams of designers, technology developers and end users. The integration team then develops the designs through iterative review with application partners and in consultation with foundation technologies. Modules are designed and tested, and as new technologies are developed or acquired, new modules are added to the repertoire. At each stage, the microsystems are tested with the biological systems of interest and iteratively improved for performance and ease of use. This tight connection between the end users of the technology and the technology developers is essential to produce effective devices with appropriate performance characteristics to address the target biological questions.

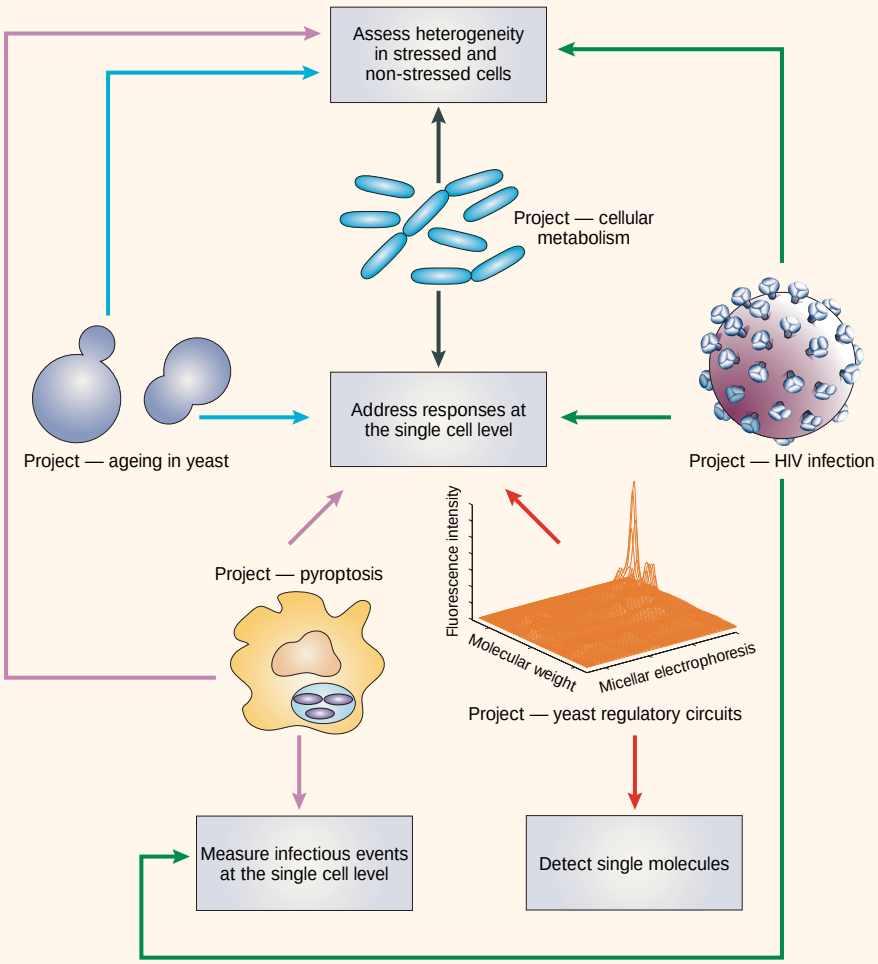

Figure 4 | **Expected MLSC outcomes.** Commonalities in outcomes for studies at the individual cell level in the MLSC. Modified with permission from REF. 52 © (2002) American Society for Biochemistry and Molecular Biology.

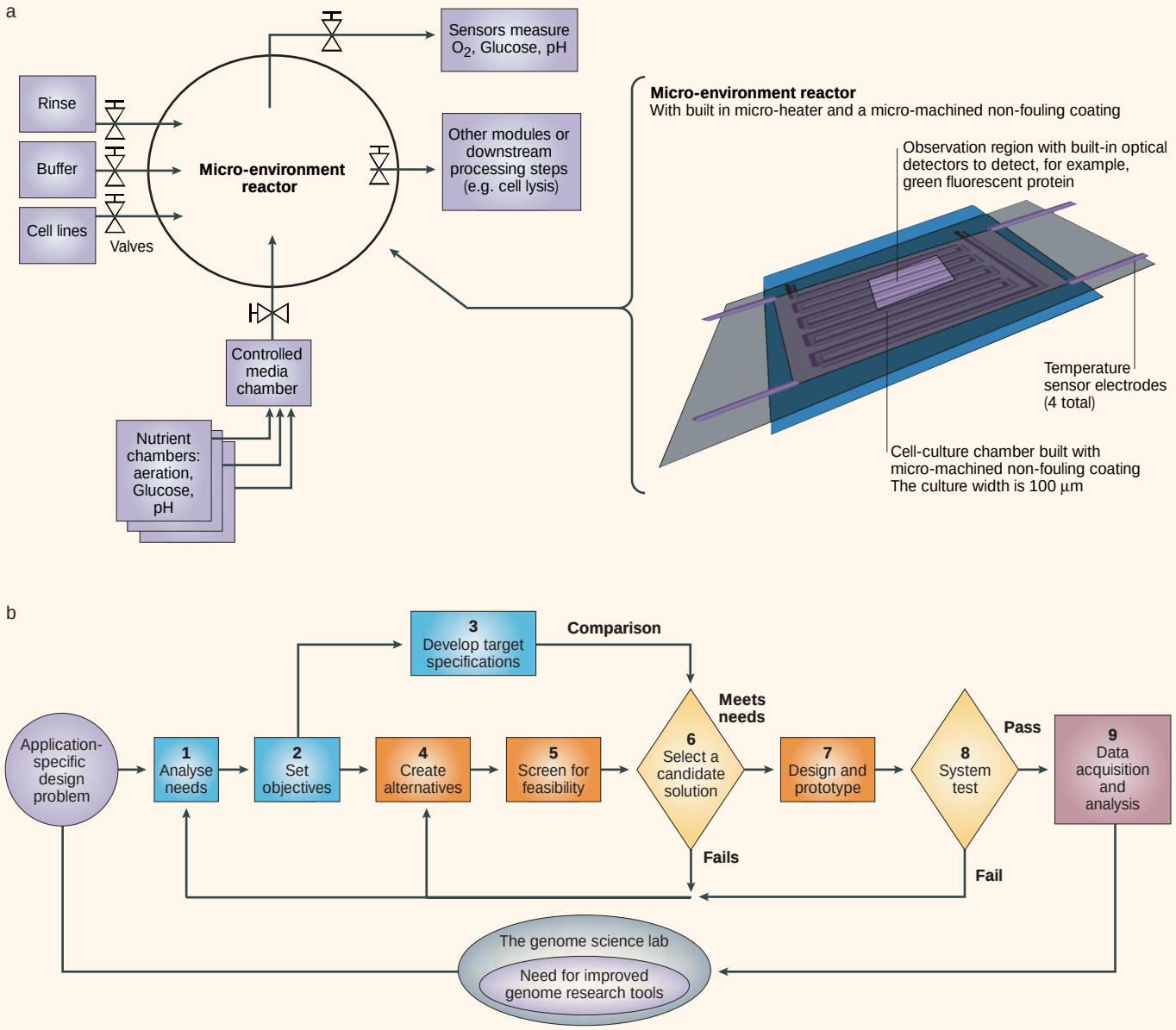

Figure 5 | **MLSC technology development. a** | Example of an integrated microsystem. **b** | Development process: systems integration and foundation technologies teams meet with applications teams to define milestones (1–3); systems integration teams develop designs through iterative review with application partners and in consultation with foundation technologies (4–7); engineering design and verification testing (4–8); and deployment of improved tools to the research laboratory (9).

Conclusions

As we move towards a detailed and quantitative understanding of cellular functions on the basis of genomics and genome-wide approaches, the need to analyse processes at the individual cell level increases. A mechanistic and predictive understanding of complex biological processes occurring at the cellular level will require studies that are aimed at the biologically important functional unit, the cell. A number of technological innovations are necessary to achieve this goal. However, recent breakthroughs in several relevant fields and an increasing number of coordinated efforts, such as that described here in the Microscale Life Sciences Center, indicate that the development and deployment of affordable, microsystem-based devices for the multiplexed, real-time, multiparameter analysis of individual cells is within our reach in the near future.

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