Perspective

Lab-on-a-Chip: Opportunities for Chemical Engineering

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Introduction

Lab-on-a-chip is becoming an increasingly familiar term used to connote the miniaturization of chemical, biological and biochemical analyses. In a broad functional sense, a lab-on-a-chip system for most (commercial) applications consists of a microchip, an instrument for control and detection, a computer with control and data analysis software, and assays and reagents. The key concept is integration and the thinking is guided by a system's viewpoint.

In one respect, a lab-on-a-chip system can be viewed as a scaled-down analog of a chemical processing plant because many process functions are similar. Undoubtedly, one key benefit in miniaturization is the drastic reduction of reagent consumption, which translates to cost savings in many applications. There are, however, two other important benefits that may be less obvious: *integration and automation*. Integration of multiple processes or functions in a single platform offers the advantages of faster analysis and sometimes novel, synergistic capabilities that may not be attainable otherwise. Automation allows more precise and reproducible operations resulting in higher data quality. All three benefits combined enable high-throughput, high quality experimentation of complex processes.

The initial concept of lab-on-a-chip, or "miniaturized total chemical analysis system," has been accredited to Manz et al. (1990), who proposed the use of integrated microfabricated devices for sample pretreatment, separation, and detection for chemical analysis. Reports of the early 1990s are often cited as pioneer studies that ignited the research in this field, but Terry demonstrated a microfabricated silicon gas chromatographic analyzer in 1975, which received very little attention from the scientific community. [For detailed history and early literature, see a recent review article by Reyes et al. (2002).] Since then, the field has been growing at a rapid pace, as indicated by numerous publications and citations, as well as the number of conferences focused on this new field.

Commercial development of lab-on-a-chip applications started in the mid-1990s by an infusion of venture capital funds, which supported the startup of many microfluidics companies. In 1999, the first lab-on-a-chip commercial product was introduced based on a collaboration between Caliper Technologies Corp. and Agilent Technologies (see http://www.chem.agilent.com/Scripts/PCol.asp?lPage=50 for details of the Agilent 2100 bioanalyzer products). In 2001, a journal called *Lab on a Chip: Miniturisation of Chemistry and Biology* was launched by the Royal Society of Chemistry to cover the growing field of research and technology development (see http://www.rsc.org/is/journals/current/loc/locpub.htm). By all early indications and the amount of current work, the lab-on-a-chip concept seems exciting and extremely viable for many applications. This technology may revolutionize the way laboratory manipulations are performed, the basic format of which has not changed significantly over the past 100 years.

So, what physically constitutes a lab-on-a-chip? First, there is a microchip at the heart of each application. In many microfluidic devices, a microchannel network is fabricated onto a substrate (glass, quartz, plastics, etc.). The channel plate is often bonded to another piece of substrate with access wells matching the ends of the microchannels, thus allowing buffers and reagents to be transported through the wells into the channels (Kopf-Sill et al., 2001). Figure 1a is an example of a microchip. Some microfluidic formats employing open channel features have also been proposed (Troian and Kataoka, 1999), although they tend to be more difficult to implement due to rapid evaporation of the nanoliter-volume fluid sample. As in the case of microelectronics, the prefix "micro" in microchips and microfluidics refers to at least one characteristic dimension of the functional feature in the micrometer range (1–100 μm). These features can be fabricated into an inorganic substrate using semiconductor processing techniques such as photolithography and etching (Harrison et al., 1992) or into a polymer substrate by polymer processing techniques such as molding, embossing, and soft lithography (McCormick et al., 1997; Kricka et al., 2002; Duffy et al., 1998).

In both liquid- and gas-phase transport applications, control of fluid, mass and energy transport is essential to laboratory operations such as reagent dispensing, mixing, incubation for chemical reactions, product purification, and detection and quantitation of reaction products. Figure 1b shows the concept of miniaturization and functional integration of these laboratory processes onto a microchip. Active control of fluid transport in microchannels can be actuated from devices external to the microchips or from pumps microfabricated inside the chip using microelectromechanical systems (MEMS) technologies (Gravesen et al., 1993). I will not discuss the pros and cons of these control methods here. Suffice it to say that without a method to actuate transport and to detect the test results, the chip itself could hardly be considered a complete lab-on-a-chip. Therefore, an instrument interfacing with the chip that provides the means to control transport and for product detection should be considered an integral aspect of a lab-on-a-chip system. Moreover, the capability of automation is impossible unless there is a computer interface with software for instrumentation control and data analysis. Finally, the lab part of lab-on-a chip necessitates

a reagent or assay component. Thus, in a broad functional sense, a lab-on-a-chip system for most (commercial) applications consists of the four physical components-a microchip, an instrument for control and detection, a computer with control and data analysis software, and assays and reagents.

Scientific and Research Challenges

Two recent articles featured in *AIChE Journal*'s Perspective column describe the benefits and research challenges of some key lab-on-a-chip functions. Jensen (1999) addresses the area of microreactors or microchemical systems. Improved thermal management, controlled reactant distribution, and fast mass-transfer rates are cited as some of the advantages of these microreactors. The real value of miniaturization lies in the exploration of new reaction pathways and finding alternative solutions to chemical manufacturing. Stone and Kim (2001) discuss the scientific challenges of microfluidics. Fluid and mass transport issues including Taylor-Aris dispersion, flow-field-fractionation for separation processes, mixing in laminar flow, flow of confined polymers, and multiphase transport are cited as current active areas of research.

Other reviews suggest the importance of integrating other lab-on-a-chip functions including on-chip sample preparation and purification, novel physical and chemical affinity separation processes, and interfacing microchips with detection methods such as mass spectrometry (Sanders and Manz, 2000; de Mello, 2001). Control of surface chemistry and interfacial interactions on channel walls is another important aspect for many applications (Mrksich and Whitesides, 1995; Zhao et al., 2001). The importance of on-chip integration of flow actuators and sensors for lab-on-a-chip devices has also been emphasized (Gravesen et al., 1993; Unger et al., 2000). Furthermore, the development of polymeric devices to take advantage of polymer processing methods for fast prototyping and mass production of low cost lab-on-a-chip applications has received a lot of attention (Effenhauser et al., 1997; McCormick et al., 1997; Duffy et al., 1998).

Undoubtedly, the development of lab-on-a-chip applications is tremendously multidisciplinary, with research and engineering opportunities straddling across chemical engineering, biology, chemistry, physics, materials, processing science, surface science, information science, and other engineering disciplines. With so many outstanding technological challenges, where does one begin to advance the lab-on-a-chip concept from R&D to commercialization?

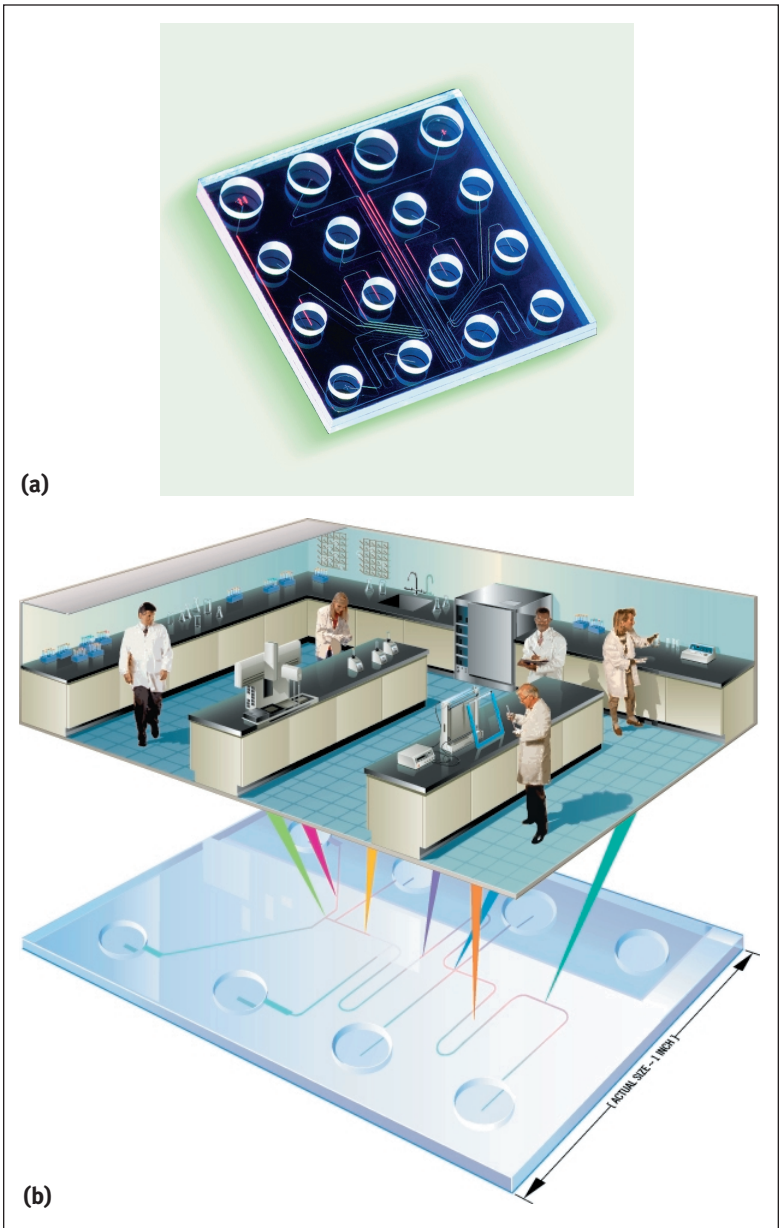


Figure 1. (a) Microfluidic microchip fabricated in glass; (b) miniaturizing and integrating laboratory processes onto a microchip device.

Application and Product Development Challenges

In developing any commercial application or product, one needs to understand clearly two issues:

(1) What are the specific features that make the product an interesting and competitive offering (does it have the features needed to do the job)?

(2) Why would people want to buy this product (is it an affordable solution to a real need)?

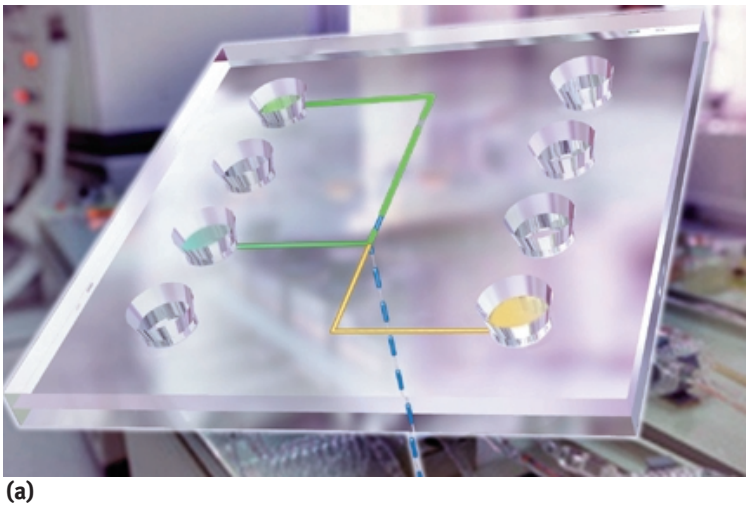
The answers to these questions help define the functional specifications of the product. For a specific lab-on-a-chip application, the overall system specifications, as well as the flow-down specifications for each of the four components (chip, instrument, software, and reagents), need to be defined specifically for that product. Functional integration of subcomponents in the microchip and system integration of all four components are the key challenges in developing this technology into viable commercial products. This point will be explained using a specific example of high-throughput screening for drug discovery, based on Caliper's technology.

In developing a successful drug to treat a specific disease, one of the first steps used by pharmaceutical companies is to screen a large library of compounds to identify lead compounds for further development (Sittampalam et al., 1997). In this initial phase of the drug development process, tens of thousands to over a million compounds are routinely tested against a "target," usually a biomolecule such as an enzyme or a receptor on a cell membrane surface that has been identified as playing an important role in controlling the disease. Currently, the standard format is a microtiter plate for storing the compounds and performing the assays. These plastic plates are about 5 in. × 3 in. in size and contain an array of wells numbering from 48 to 1,536, with the most commonly used ones being 96- and 384-well formats. Most high-throughput screening facilities are equipped with laboratories full of robots and automation equipment to handle these plates, moving them from station to station for reagent dispensing, mixing, reaction incubation and readout of the assay results mostly based on fluorescence or radiometric detection methods.

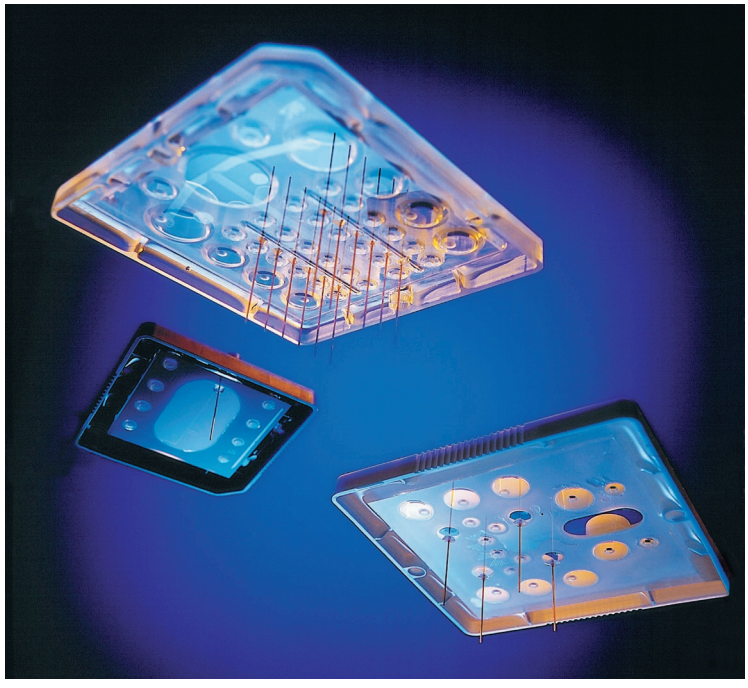
System design and integration

High-throughput screening can benefit readily from the miniaturization, integration, and automation offered by lab-on-a-chip technologies. In developing such a product, one system consideration is deciding how to partition disposable and nondisposable components. Some researchers in the field propose integrating many functions, including micropumps for flow actuation and detection microsensors, onto the chip itself as the ultimate goal of integration for lab-on-a-chip applications. However, from the economics perspective, the chip may become too expensive to manufacture if it is considered disposable or semidisposable (e.g., a single chip is used for up to a day for running tens of thousands of experiments.) Consequently, one reasonable choice is to have passive channels on a chip, with flow actuation mechanism and detection optics incorporated in the instrument, which is not disposable and can be less cost-sensitive.

Devising a lab-on-a-chip system for high-throughput screening requires developing a suitable world-to-chip interface capable of automating compound sampling from the microtiter plate format. This interface, integrating the chip to the existing sample storage, must be manufacturable, have low dead volume such that sample cross-contamination is minimized, and be scaleable to increase throughput via parallel processing. One solution is a sipper chip format that uses a capillary connection to allow the planar microchannel network to communicate fluidically to the outside world. A few nanoliters of each compound in solution can be brought onto the chip serially, and adjacent compounds are separated by a buffer spacer to keep the samples spatially distinct. Figure 2a illustrates the concept of serial compound accession, and Figure 2b shows the sipper chip family, from 1 to 12 sipper connections in one single chip.



(a)



(b)

Figure 2. (a) Automated compound sampling through a sipper capillary into a microchip device with blue sample plugs separated by clear buffer spacers as they are brought onto the chip serially; (b) sipper chip family including 1-, 4- and 12-sipper chips.

Functional integration on chip

One level down from the overall system, the detailed functional specifications for chip design are determined by the specific assays to be performed in the chip. The number of questions may surprise the nonexperts. The following is a list of the questions we need to answer for each assay type:

- What are the characteristics of the reagents and products for the assay?
- How should the fluids and reagents from the sipper and in the microchannels be transported?
- In what order must the reagents be added and what are the mixing requirements?

• What is the chemistry or biochemistry involved in the reaction? How long does the reaction mixture need to incubate and at what temperature? What are the design criteria for the reactor?

• Do the products need to be separated from the reactants before detection? If yes, what separation method should be used and what are the optimal conditions?

• What are the mass and volumetric flow rates in and out of the various unit operations in the chip? Is the yield of products high enough to meet detection requirements?

• How much heat is generated or required in each unit operation and what method should be used to manage the heat flow in and out of the chip?

• For each unit operation, is it continuous flow or batch process? If mixed mode processes are used, how do we integrate the different processes?

• What are the key scaling parameters in the design? How do we take bench top results and scale it down for microfluidic processing?

• When all the necessary unit operations are integrated together on chip, what is the overall sample throughput? Does it still make a viable product?

These issues are similar to the ones that would be asked in the beginning of a more conventional chemical process plant design project. It is then apparent that answering these questions most definitely needs the analytical skills in mass and energy balance, transport phenomena, reaction kinetics, separation processes, dimensional analysis and scaling laws, unit operations, process control, economic analysis, and other subjects found in most chemical engineering curriculum.

To complete my example on functional integration on chip, I will use kinase enzymatic assays for high throughput screening to illustrate the development process. Kinases are an important class of enzymes that the body uses to regulate many biochemical pathways and are, therefore, very interesting pharmaceutical targets. Functionally, a kinase takes a phosphate group from adenosine triphosphate (ATP) and puts it onto a peptide (called "substrate") containing a specific sequence of amino acids that the kinase recognizes, thereby turning it into a product that is more negatively charged (this process is called "phosphorylation"). A kinase screening assay is relatively difficult to run on the conventional microtiter plate format, because no one has yet been able to find a way to make the assay "fluorogenic," meaning that the fluorescence signal of the labeled substrate changes as it turns into a product. Without a changing fluorescence signal, one must separate the product from the substrate before detecting the extent of reaction. This requires chemically or physically stopping the reaction, then using a separation means (such as a chromatographic column) to differentiate the product from the substrate, and then measure the product or substrate concentration. In a high-throughput environment in traditional formats, these steps are difficult to implement.

To develop a microfluidic kinase assay for screening, we can take advantage of the fact that the substrate and product have different electrophoretic mobilities because the product is more negatively charged than the substrate. Following reaction incubation, an electrophoretic separation step can be used to quantify the rate of reaction by comparing the ratio of the substrate to product signal. We can use the approach of inject-and-separate using a channel intersection as a virtual valve that many have demonstrated on chip, in which an aliquot of reaction mixture can be injected into a separation channel using an injection scheme such as pinched injection or gated injection (Jacobson et al, 1994). Under adequate electric field strength and separation time, the resulting signal shows two separate peaks because the two labeled species travel at different velocities under the applied field. However, inject-and-separate is inherently a batch process, which makes integrating it into the continuous flow reaction incubation step more difficult. At the very least, one would need to monitor when each sample reaction mixture arrives at the injection intersection to time the sample injection into the separation channel.

To avoid the complexity of timing of injections, we consider the alternative of turning the separation process from batch to continuous flow. In this case, we use the injection of sample into the sipper as the injection step for the reaction, as well as for the separation. The potential drawback is that the sample plug injected through the sipper is usually much larger (of the order 1mm in plug length) than that injected through a microchannel intersection (of the order of 10–100 μm), potentially reducing the sample throughput rate because of the need for longer separation time, which has a strong dependence on the sample plug length (Bousse et al., 2000). As demonstrated by experimental data and by simulations, however, the separation resolution criterion in the continuous flow method is less stringent than that for the batch inject-and-separate method. Thus, the requirement for separation time turns out not to be overly cumbersome for this continuous flow method.

A single-sipper version of the chip design for the continuous flow kinase assay is shown in Figure 3a. Solutions of the kinase enzyme and labeled substrate are placed in the chip reagent reservoirs and fed into the reaction channel, mixed with the compound sipped from the microtiter well, and the three reactants are allowed to incubate for a specific length of time before the mixture flows into the separation channel. One requirement for the continuous flow reactor is that the reactants must be co-located as they move along the channel. Therefore, pressure-driven flow, not electrokinetic flow, is chosen as the actuation mechanism. In the simplest case, a steady, reduced pressure is applied in the waste well (upper left well in Figure 3a), and all other reagent reservoirs, electrode wells, and the tip of the sipper are at atmospheric pressure conditions. The reduced pressure at the waste well continuously drives all reagents into the reaction and separation channels.

Two other important considerations for the reactor design are to ensure adequate mixing of the reactants and to minimize sample broadening due to dispersion to maximize sample throughput. The nonuniform velocity gradient generated by pressure-driven flow causes the samples to disperse, and this dispersion dictates the spacing of neighboring samples and, therefore, the throughput of the analysis.

In Stokes flow characteristic in these microchannels, mixing time (t) is dictated by thermal diffusion described approximately by the Einstein diffusion equation

$$t = w^2/2D$$

in which w is the channel width and D is the molecular diffusion coefficient. This means that mixing time can be reduced by incorporating a narrower width in the initial portion of the reaction channel. A factor of two reduction in the channel width results in a factor of four reduction in mixing time, so one should design the mixing part of the reaction channel to be as narrow as possible. The question is whether diffusion mixing in a reasonably sized channel width is adequate for this assay. Or do we need to utilize additional mixing mechanisms? An analysis of the enzyme kinetics equation indicates that it is important for the compound (from the sip-

per) and the substrate (from the on-chip well) to be adequately mixed across the channel width; whereas, the concentration profile of the enzyme is not as important, as long as the local concentration of enzyme does not exceed the stoichiometric concentration of the substrate. Since we normally use at least an order of magnitude higher concentration of the substrate than enzyme, the mixing time dictated by diffusion of substrate only is quite reasonable and no additional mixing scheme is usually required.

Within the range of flow velocity often used in these microchannels (on the order of 0.01 to 1 cm/s), the dispersion is less dramatic than in larger flow channels because molecular diffusion transverse to flow is an important transport means to mediate dispersion along the flow direction in these length scales. Molecules diffuse across fast and slow streamlines in a short period of time compared to the characteristic time for flow. Dispersion under these conditions is described by the Taylor-Aris models, in which the design parameters dictating dispersion are well understood (Brenner and Edwards, 1993). In the steady-state condition, the Taylor-Aris dispersion coefficient (D_{TA}) is proportional to the squared of the averaged flow velocity (u) and channel depth (d)

$$D_{TA}=Cu^2d^2/D$$

with the proportionality constant C determined by the channel cross-sectional geometry. The broadening of the sample (σ) varies with square root of time (t)

$$\sigma=[2(D+D_{TA})t]^{1/2}$$

Usually, the reaction incubation time requirement dictates the choice of t. Both channel geometry and velocity are viable parameters for minimizing dispersion.

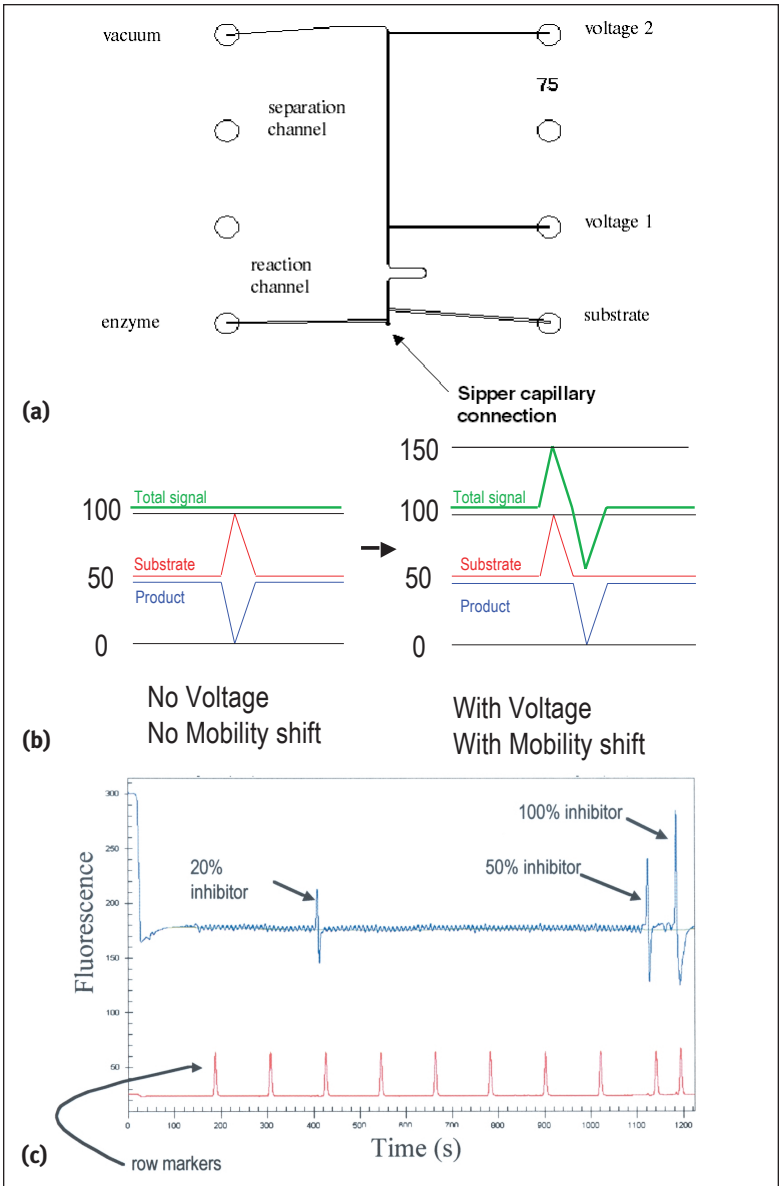


Figure 3. (a) Chip layout of a single sipper designed for high-throughput screening of kinase assay with on-chip reaction incubation and electrophoretic separation; (b) expected fluorescence signal with and without an applied electric field in separation channel (peak-and-dip signal on the right is characteristic of a kinase inhibitor); (c) measured fluorescence of Protein Kinase A screening with samples brought up from a microtiter plate spiked with 3 levels of inhibitor potency (the row markers are used as a real-time process monitor for mapping sample arrival time to plate location).

Now that we have considered fluid and mass transport, reactor design, and separation mechanism, what do we expect the fluorescent signal to look like when an active compound is sipped into the channel? If the compound is an inhibitor that slows down the turnover rate of the kinase, then the substrate concentration will be higher locally and the product concentration will be lower than in the buffer region, as illustrated in Figure 3b. Without an electric field, the total fluorescence intensity would stay the same since the brightness of fluorescence for both species is identical. When the mixture reaches the separation channel, however, the electric field retards one of the components, resulting in a peak-dip signature (or dip-peak depending on the polarity of the electrodes). Figure 3c shows actual kinase assay data with known inhibitors placed in the microplate wells during the screening run of Protein Kinase A. The amplitude of the peak, calibrated against a control 100% inhibitor, determines the potency of the compound. It is worth noting that from the standpoint of assay robustness, we have implemented two types of process monitoring parameters for each 384-well plate. One is the control 100% inhibitor to calibrate the fluorescence signal at zero enzyme activity needed for base line signal subtraction. Another is the periodic dye sip to monitor flow rate (lower trace of Figure 3c) ensuring that the mapping of compound arrival time to position in the microtiter plate is of high fidelity.

This example that I just presented is a real commercial application to be introduced in a 4-sipper format, and it helps to illustrate two points. First, functional integration for lab-on-a-chip applications is

very much application specific, and second, chemical engineering curriculum provides an excellent training for engineers to take on the exciting challenges in system and functional integration of product development in this new and promising field of lab-on-a-chip.

Opportunities for Chemical Engineering

At this stage of development, research and engineering opportunities for lab-on-a-chip applications appear almost *limitless*. The potential comes not only from the multidisciplinary nature of the research questions, but also from the promise of a revolutionary change in laboratory science and its potential impact in a number of important areas including analytical chemistry, chemical synthesis, cell biology, molecular biology, drug discovery, genomics, proteomics, diagnostics, environmental monitoring, and national security. The fundamental training in scientific and engineering principles of fluid mechanics, heat and mass transfer, reaction chemistry, scaling laws, etc. that chemical engineers have always received is perfect for researchers and developers in the emerging field of lab-on-a-chip. As opportunities for chemical engineering are evolving and expanding, curriculum electives in colloids and surface science, physical chemistry, biochemical and cellular technology, and material processing further enhance chemical engineers' value to the field.

The preparation of future chemical engineers for the challenges in integration-both in the sense of integrating the subcomponents for an application, as well as of integrating into multidisciplinary teams-is a topic that, I would argue, deserves some level of attention and discussion. Integration is not an issue traditionally addressed explicitly in the chemical engineering curriculum. One interesting proposal is to explore the value of exposing students to team-oriented design projects that foster collaboration with people outside the chemical engineering boundaries, involving either faculties in other academic departments or researchers in the industry.

In summary, I believe that the enabling features and capabilities that integrated microfluidic devices and systems bring to laboratory science is an exciting area in which chemical engineers can make significant contributions. I would argue that the chemical engineering curriculum is ideally suited to prepare engineers to take on the challenges of integration in the development of lab-on-a-chip applications. Chemical engineering students are trained in mass and energy balance, transport phenomena, chemistry, reaction kinetics and reactor design, thermodynamics, separation processes, dimensional analysis and scaling laws, process control, process optimization, unit operations, plant design, and economic analysis. *All* of these subjects are relevant for most lab-on-a-chip applications. Creating novel and useful lab-on-a-chip products uses their fundamental skills and can potentially have a tremendous impact on accelerating the pace of research and improving the efficiency in many areas of applications in this rapidly growing field.

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