TECHVIEW: MICROFLUIDICS

Microfluidic Diffusion-Based Separation and Detection

Bernhard H. Weigl* and Paul Yager

Recent advances in device miniaturization have led to the development of integrated microfluidic devices, so-called labs-on-a-chip (*1*, *2*). In these tiny microchips etched with grooves and chambers, a multitude of chemical and physical processes for both chemical analysis and synthesis can occur. These devices, also known as micro–total analysis systems (μTAS), can be mass produced in silicon by techniques similar to those used in the semiconductor industry, or for even lower cost, they can be made out of plastics by using casting, cutting, and stamping techniques. They offer many advantages over traditional analytical devices: They consume extremely low volumes of both samples and reagents. Each chip is inexpensive and small. The sampling-to-result time is extremely short. In addition, fluids flowing in microchannels exhibit unique characteristics ("microfluidics") that allow the design of analytical devices and assay formats that would not function on a macroscale.

A true μTAS will perform all analytical functions including sampling, sample pretreatment, separation, dilution, mixing steps, chemical reactions, and detection in an integrated microfluidic circuit. Microfluidic chips that perform all of these individual functions have been demonstrated (*3*–*5*), but they have not all been integrated in one device. Existing μTAS technologies work very well for highly predictable and homogeneous samples common in genetic testing and drug discovery processes. One of the biggest challenges for current μTAS, however, is to perform analysis in samples as complex and heterogeneous as whole blood or contaminated environmental samples.

Separation of particles from soluble components is usually done by filtering or centrifugation. None of these methods is suitable for integrated microsystems. Efficient centrifugation requires that the sample be placed at some distance from the center of rotation in order to achieve a high enough centrifugal force, which is usually not practical in a microfluidic system. Filtering requires membranes, which can clog, and these are difficult to mass-manufacture as part of a microfluidic circuit.

In channels in which either width or height is less than ~200 μm (typical of most microfluidic devices), liquids that flow slowly follow predictable laminar paths characteristic of low Reynolds numbers. (The Reynolds number is a nondimensional parameter relating the ratio of inertial to viscous forces in a specific fluid flow configuration.) This allows two or more layers of fluid to flow next to each other without any mixing other than by diffusion of their constituent molecular and particulate components. Aqueous flow streams in a microchannel are hydrodynamically similar to very viscous, oil-like streams in a significantly larger channel.

Microfluidic systems have distinctive properties as a result of their small dimensions: (i) aqueous flow is generally laminar, not turbulent; (ii) diffusion is an efficient process for mixing the dissolved contents of two or more fluids; and (iii) particles can also be separated by diffusion according to their size. For example, at room temperature in an aqueous solution, a spherical molecule with a molecular weight of 330 (for example, a small organic dye molecule) takes 0.2 s to diffuse 10 μm, whereas a larger particle with a diameter of 0.5 μm (for example, a small bacterium) would require about 200 s to cover the same distance (*6*).

The so-called T-Sensor, a μTAS component that combines separation and detection functions, is based on these properties. A T-Sensor system has been demonstrated (*6*) in which a sample solution, a receptor (indicator) solution, and a reference solution are introduced in a common channel (Fig. 1). The fluids interact during parallel flow until they exit the microstructure. Large particles such as blood cells do not diffuse significantly within the time the flow streams are in contact. Small particles such as H^+, Na^+, and small molecules diffuse rapidly between streams, whereas larger polymers diffuse more slowly and equilibrate between streams further from the point of entry to the device. As interdiffusion proceeds, interaction zones are formed in which sample and reagents may bind and react. Typically, an indicator changes color or fluorescence intensity upon interdiffusion and reaction with analyte molecules. Although intensity, width, and shape of the interaction zone between sample and indicator may yield information about analyte concentration, the ratio of a property such as fluorescence of both interaction zones (sample-indicator and reference-indicator) is usually used to determine the concentration of the analyte. Such a ratio can be largely free of cross-sensitivities to other sample components and instrumental parameters, and enables

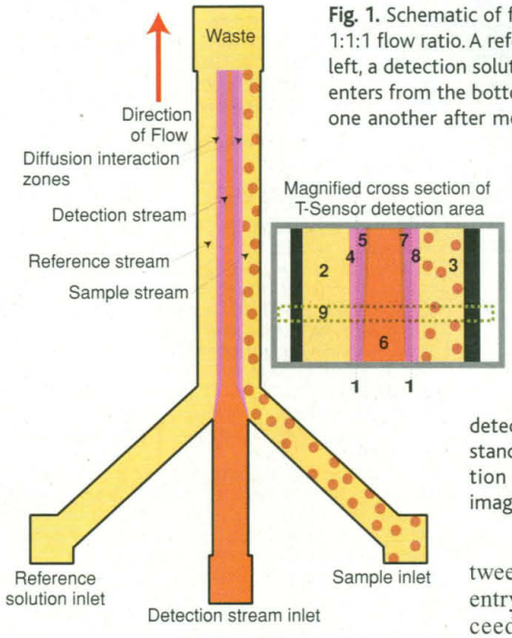

Fig. 1. Schematic of flow and diffusion within the T-Sensor at a 1:1:1 flow ratio. A reference solution enters the device from the left, a detection solution from the middle, and a sample stream enters from the bottom right port. All streams flow adjacent to one another after merging at the T junction of the central detection channel. The detection channel stream exits at the port shown at the top. The magnified schematic to the right of the channels shows (**1**) original flow boundaries, (**2**) reference stream, (**3**) particle-laden sample stream, (**4**) diffusion of detector substance into reference stream, (**5**) diffusion of reference analyte into detection stream, (**6**) detection stream, (**7**) diffusion of sample analyte into detection stream, (**8**) diffusion of detector substance into sample, and (**9**) detector cross-section (linear detector array) or area (such as imaging CCD).

B. H. Weigl is at Micronics, Inc., Redmond, WA 98052, USA. P. Yager is in the Department of Bioengineering, University of Washington, Seattle, WA 98195, USA.

*To whom correspondence should be addressed. E-mail: bernhardw@micronics.net.

essentially calibration-free sample analysis without the need for external sample preprocessing or blood cell removal. If an indicator solution is used in the detection solution, the diffusion interaction zones will be optically detectable. The positional variation in intensity of that signal is a complex function of the concentration of the indicator and analyte (*7*). However, it is straightforward to calibrate the optical response to analyte concentration.

Figure 2 shows images of the diffusion interaction zones in a T-Sensor during a series of albumin assays with increasing concentrations of that analyte. The left line is the interaction zone between reference and detection solutions, and the right line shows the interaction between indicator and sample solutions.

Single-frame video or charge-coupled device (CCD) images of the detection channels for each sample are digitized and processed. A wealth of data can be gained from determining the absorption or fluorescence profile across one or more portions of the detection channel. The cross-sections are typically divided into several zones, including reference background, reference interaction zone intensity, detection solution background, sample interaction zone intensity, and sample background. By applying custom algorithms comparing intensities and positions of diffusion interaction zones, these parameters yield essentially calibration-free analytical concentration values independent of variations in experimental conditions. It is possible to compensate for effects such as variations in flow-cell geometry, temperature-dependent reaction kinetics, light source stability, instabilities in the optical system and detection electronics, as well as fluid parameters such as turbidity, color, concentration of detection chemistry, cross-sensitivities to other sample components, viscosity, and flow speed.

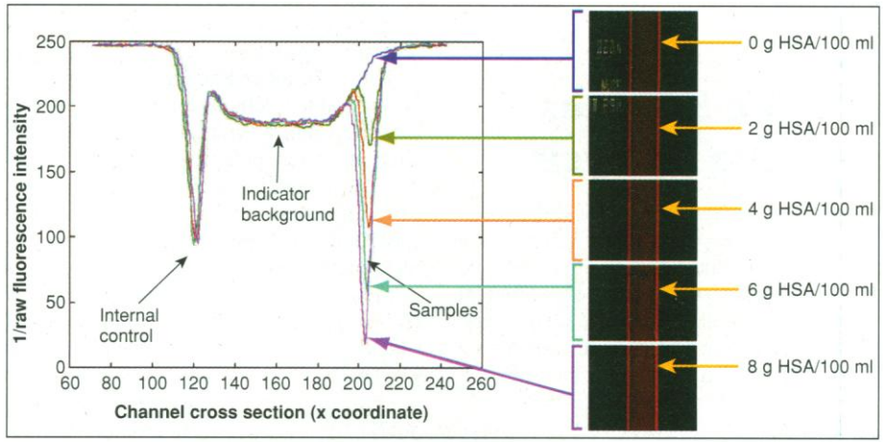

Fig. 2. Fluorescence micrographs of a detection channel section containing control, indicator, and sample streams during a determination of human serum albumin (HSA, right side of the image) and a graph displaying the corresponding light intensity profiles across the width of the channel. Many analytic parameters can be derived from these cross-section profiles, including (i) reference and sample solution fluorescence or absorption background, (ii) diffusion interaction zone intensity, width, and diffusion profiles for both sample and reference solutions, (iii) detection solution fluorescence or (iv) absorption background, and (v) x-location of reference and sample interaction maximum intensity. Parameters (i) through (iv) are used for calibration, control, and for calculating the concentration of the analyte in question; parameter (v) yields information about the relative viscosities of the streams in the channel.

Signal strength is an inherent problem of all optical detection in microchannels, a result of typically very small optical pathlengths. Because all flow in a T-Sensor is laminar, and reagents and sample are constantly renewed, images can be integrated over time for greater sensitivity without fear of photobleaching, reagent degradation, separation membrane clogging, and other problems typical of traditional sensor systems.

Comparison of referenced versus nonreferenced data for a typical T-Sensor experiment (Fig. 3) illustrates the significantly enhanced accuracy of the sample concentration determination when the sample fluorescence intensity is normalized to the fluorescence intensity of the reference channel.

To date, T-Sensor assay feasibility has been demonstrated for a variety of clinical parameters such as blood pH and oxygen, electrolytes, proteins, enzymes, and drugs through the use of detection methods ranging from fluorescence and light absorption to voltammetry (*6–9*). Of particular interest are also novel immunoassay formats that use the diffusion separation feature of T-Sensors to isolate and detect bound and unbound antibody-antigen complexes. In addition, monitoring signal intensities along the T-Sensor detection channel (in-flow direction) provides a means for looking at the kinetics of a reaction, thus allowing kinetic diagnostic reactions to be measured not as a function of time, but of distance from the starting point of the diffusion interaction.

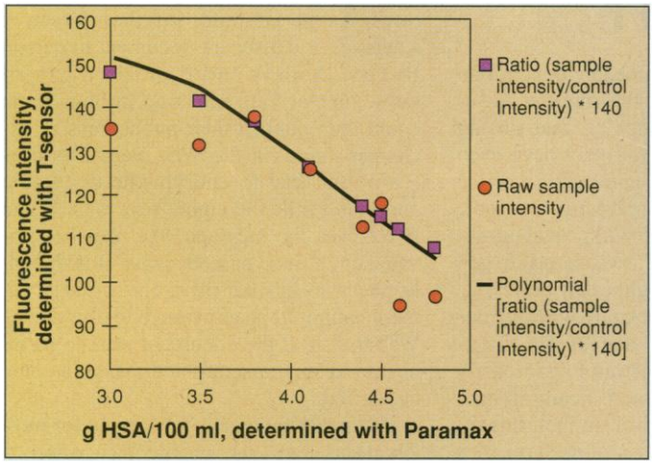

Fig. 3. Comparison of referenced versus nonreferenced sample concentration data for a typical HSA T-Sensor determination. The sample concentration was derived from the ratio of (sample interaction zone intensity)/(reference interaction zone intensity), whereas raw sample intensity was obtained directly from the sample interaction zone intensity.

References

1. R. F. Service, *Science* **282**, 399 (1998).
2. M. A. Burns *et al.*, *Science* **282**, 484 (1998).
3. A. Manz, *J. High Resolut. Chromatogr.* **16**, 433 (1993).
4. J. P. Brody and P. Yager, in *Solid-State Sensor and Actuator Workshop*, Hilton Head Island, SC (Transducers Research Foundation, 1996).
5. D. J. Harrison, *Science* **261**, 895 (1993).
6. B. H. Weigl, M. Holl, D. Schutte, J. P. Brody, P. Yager, *Analytical Methods & Instrumentation*, μTAS96, November 1996, Basel, Switzerland (Kluwer, Dordrecht, Netherlands, 1996).
7. A. Kamholz, B. H. Weigl, B. Finlayson, P. Yager, in preparation.
8. B. H. Weigl, presentation only, CRC Conference on Microfabrication for Biomedical Applications, October 1997, San Jose, CA.
9. R. B. Darling, P. Yager, B. H. Weigl, J. Kriebel, K. Mayes, in *Micro Total Analysis Systems '98*, 12 to 18 October 1998, Banff, Canada, D. J. Harrison and A. Van Den Berg, Eds. (Kluwer, Dordrecht, Netherlands, 1998), p. 81.