SLIPCHIP, CHEMISTRODE, AND DROPLET-BASED MICROFLUIDIC TECHNOLOGIES: FROM BASIC SCIENCE TO APPLICATIONS
Wenbin Du, Liang Li, Feng Shen, Weishan Liu, Kevin P. Nichols, and Rustem F. Ismagilov*
University of Chicago, USA

ABSTRACT
This presentation will provide an overview of our recent work in development of microfluidic technologies, including SlipChip, Chemistrode, and continuation of the work on plug-based microfluidics. These technologies enable two lines of investigation: 1) for basic science, they advance our understanding of dynamics of complex reaction networks in space and time, 2) for applied science, they are advancing point-of-care diagnostics and laboratory analysis under resource-limited settings. Examples include digital nucleic acid analysis for quantitative molecular diagnostics (e.g. quantification of the viral load), immunoassays to quantify small numbers of proteins, highly multiplexed PCR and immunoassays, and analysis of cells and their interactions.

KEYWORDS: PCR, immunoassay, single molecule, microbial communities, digital PCR, multiplexed PCR, chemistrode, SlipChip

CHEMISTRODE
The chemistrode (Figure 1) is a droplet-based microfluidic device that relies on two-phase laminar flow to collect chemical signals with high spatial and temporal resolution [1-4]. Most biological processes rely on molecular signals rather than electrical signals to coordinate a series of events, but electrochemical approaches are widely used because of the ease with which electrochemical signals can be observed. The chemistrode addresses the need for an analogue to an electrode that uses molecular rather than electrical signals. We have demonstrated chemistrodes that can sample secretions from tissue in an isolated well, and needle-like chemistrodes that can sample soil suspensions [2].

The output of the chemistrode can be analyzed by a variety of techniques, including single molecule spectroscopy. The droplet-based nature of the chemistrode allows each droplet ("plug") to be split into identical daughter droplets that can then be analyzed using multiple assays that may be incompatible on a single sample, such as functional assays and genetic analysis [2]. However, we have found that the analysis of a large number of small-volume plugs is still a limiting step in the use of the chemistrode. The chemistrode provides one example of a general challenge of analyzing many nanoliter samples in parallel. To address this challenge, we developed the SlipChip.

SLIPCHIP
The SlipChip [5-10] (Figure 2) is a simple microfluidic platform that can perform massively parallel manipulation of fluid volumes. The SlipChip is constructed of two plates that are separated by a thin layer of inert lubricating fluid, e.g. air, fluorinated oil, or mineral oil. SlipChip can encode, as a sequence of wells and ducts imprinted in the two plates, essentially any program to manipulate fluid volumes. It relies on simple slipping of the two plates relative to one another.

Figure 1: Chemistrode to deliver and record multiple molecular signals with high temporal and spatial resolution for off-line analysis by multiple analytical methods [1]. (A) A conceptual schematic drawing. (B) Time-lapse bright-field images (side view) of an incoming stimulus plug (droplet) merging with the wetting layer above a glass surface and the formation of a response plug as the fluid exits the wetting layer.

Figure 2: Schematic of the SlipChip showing a three-dimensional view (top) and a cross-sectional view along the dashed line in (a) (bottom). The top plate of this SlipChip design contains wells for the sample, the bottom
to execute the program (Figure 2). A solution can fill the wells, for example, by flowing through a fluidic path created by overlapping wells one plate with ducts in the other. Slipping of wells away from the ducts compartmentalizes small volumes of the solution. The highly paralleled and multiplexed nature of the SlipChip make it amenable to small-volume processing including single-molecule detection of molecular targets and multi-step processing upstream and downstream of detection. The SlipChip can be used for small-volume analysis in immunoassays [8] (Figure 3), protein crystallization [5-7] (Figure 4), digital PCR [9] (Figure 5), and multiplex PCR [10]. For example, immunoassays are a class of analytic techniques widely used in biological research, but bottlenecks associated with small-volume processing must be overcome to perform immunoassays efficiently in small volumes. We have demonstrated a heterogeneous immunoassay on nanoliter droplets in SlipChip [8] (Figure 3) that can be used to analyze samples that are collected using the chemistrode.

![Figure 3](image1.png)

**Figure 3:** Heterogeneous immunoassays with nanoliter samples on SlipChip [8]. Schematic of the operation of the bead-based immunoassay in SlipChip (cross-section). The sample is loaded into wells in the bottom plate, and is sequentially slipped into contact with wells in the top plate containing the capture antibody: bead and detection antibody: enzyme, washing buffer, and the fluorogenic substrate. Magnets were used for mixing.

![Figure 4](image2.png)

**Figure 4:** Performing both microbatch and free interface diffusion (FID) crystallization methods to screen different reagents and different concentrations in a composite SlipChip [7]. Crystallization of dihydrofolate reductase/thymidylate synthase from Babesia bovis in both microbatch (A) and FID (B). (C) The structure of the protein was determined at 1.9 Å resolution.

For applications that require intensive multiplexing, such as protein crystallization [5-7], multiple crystallization conditions (reagents, mixing ratio, and crystallization method) can be screened on a single SlipChip [6,7] (Figure 4) to quickly identify protein crystallization conditions with high throughput. We designed a device that combined both microbatch and free interface diffusion methods for crystallization on the same chip. The SlipChip has the added benefit of using very small volumes of sample (i.e. 4 μL of sample for 160 experiments on a single chip), a feature important in protein crystallization where the protein sample may be in limited supply.

Digital PCR is a powerful method to quantitatively detect rare cells and count cells, DNA, and RNA. This method relies on the single-molecule sensitivity of PCR, and we demonstrated a simple, inexpensive platform for digital PCR based on the SlipChip [9]. The device was designed to increase the density of wells and used overlapping elongated
wells to form the fluidic path. This design prevented sample from being wasted in the ducts and reduced the pressure drop along the device, allowing all 1280 wells to be filled rapidly (Figure 5).

Figure 5: Digital PCR on a SlipChip [9]. A) Assembled digital PCR SlipChip. Black solid line indicates the top layer and blue dotted line shows the bottom layer. B) Sample was loaded into the SlipChip through the continuous fluidic path by pipetting. C) The bottom plate was slipped up, and the wells on top and bottom plates overlapped to form individual compartments for digital PCR. D) Bright field microphotograph shows the formation of compartments. Red food dye was used in this experiment. E-F) Digital PCR on the Slipchip with a serial dilution of Staphylococcus aureus DNA template.

The SlipChip is also attractive for multiplex PCR [10], as it enables high-throughput screening that could be applied to clinical diagnostics. The multiplex PCR SlipChip was designed with circular wells in the bottom plate containing pre-loaded primers under lubricating oil and square wells in the top plate containing the aqueous sample. This design overcame issues associated with thermal expansion during thermal cycling. Each circular well could contain a different pre-loaded primer, allowing us to screen up to 384 different primer pairs with less than 30 nL of sample. To test the device, we pre-loaded 20 different primer pairs onto different regions of the SlipChip to identify 16 different pathogens. One additional primer pair was pre-loaded onto four regions of the chip for a positive internal control, and four other regions were left empty for a negative internal control. In our experiments, all positive controls showed increase in fluorescence intensity and no negative controls showed increase in fluorescence intensity. The multiplex PCR SlipChip was able to correctly identify methicillin-resistant S. aureus, methicillin-sensitive S. aureus, C. albicans, P. aeruginosa, and E. coli.

MICROBIAL COMMUNITIES

Microbial communities are responsible for a wide range of functions, including bioremediation of contaminants and maintaining colon homeostasis within the human body. While metagenomics can provide information on the genetic diversity of these communities, they do not provide live cells to study and analyze. Furthermore, many microbes found in the environment have not been cultivated under laboratory conditions. We are using droplet-based technologies to stochastically confine single cells from mixtures [2] and have used well-based technologies to study the effects of confinement on quorum sensing in single cells [11], a density-dependent behavior of microbes. We have also developed microfluidic devices to study community interactions [12] and determined the necessity of spatial structure in maintaining a microbial community. Recent work includes using microfluidic techniques to design spatially structured synthetic microbial communities to perform desired functions such as bioremediation of a mixture of contaminants [13]. New approaches will be discussed to capture single cells of interest and to measure the interactions among different bacterial species to harness microbial functions.

CONCLUSION

We are in the process of expanding the basic science and applications that we have discussed above. We are investigating the integration of sampling and analysis by linking the chemistrode and the SlipChip. In the SlipChip, we are investigating isothermal nucleic acid amplification methods, expanding the dynamic range of digital devices, improving the sensitivity of immunoassays, and performing quantitative assays with visual readout. These improvements to the basic microfluidic platforms that we have developed would greatly increase the impact of these platforms and allow for quantitative analysis to be performed outside of high-tech laboratories and research centers. The applications of these microfluidic platforms to resource-limited and point-of-care settings would greatly impact healthcare worldwide. The microbial world remains poorly understood and poorly utilized, and new technologies for capturing cells and measuring their interactions will open exciting opportunities.

ACKNOWLEDGEMENTS

The work described in this presentation was supported by the NIH Director’s Pioneer Award, part of the NIH Roadmap for Medical Research (1 DP1 OD003584), the Office of Naval Research grant No. N00014-08-1-0936, the NSF CRC grant CHE-0526693, the Camille Dreyfus Teacher-Scholar Awards program, the Chicago Center for Systems Biology (funded by the National Institute of General Medical Sciences at the NIH). We thank Heidi Park for contributions to writing and editing this manuscript.
REFERENCES


CONTACT

*R.F.I, r-ismagilov@uchicago.edu; webpage: http://ismagilovlab.uchicago.edu