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Microfluidics in Systems Biology – Hype or Truly Useful?

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Abstract

Systems biology often relies on large-scale measurements and to build models to understand how complex biological systems function. Microfluidic technology has been touted as a tool for high-throughput experiments and has been a valuable tool to some systems biology research. This review focuses on applications where microfluidics can enhance experimental sensitivity and throughput, particularly in recent development in single-cell analyses and analyses on multi-cellular or complex biological entities. We conclude that microfluidics is not necessarily always useful for systems biology, but when used appropriately can greatly enhance experimentalists' ability to measure and control, and thereby enhance the understanding of and expand the utility of biological systems.

Graphical abstract

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Introduction

One goal in systems biology is to measure and model how molecular or cellular networks can generate phenotypic characteristics in complex biological systems [1][2]. An important aspect of the experimental work is to make large-scale systematic measurements on phenotypes and parameters in order to build predictive models and subsequently test the hypotheses predicted by these models [3]. While sequencing techniques and genome manipulation techniques (e.g. cloning and gene editing) have greatly advanced in the last decade with much reduced complexity and cost [4][5], significant challenges remain in manipulating samples and performing phenotypic characterizations, especially at the single-cell level and in complex multicellular systems. Furthermore, in some cases, system-wide measurements are still difficult, if not impossible, to make due to the low-throughput nature of some conventional experimental tools. Some of these difficulties could potentially be addressed by microfluidic technologies developed and popularized in the last decade [6]. The advantages of microfluidic systems include the micro-scale features that match that of many biological systems (e.g. single-cells, small organisms or tissues) and laminar flow, which enables precision delivery of fluids [7][8]. Most microfluidic systems are also compatible with microscopy tools. In addition, miniaturization allows for many parallel experiments on the same chip, while automation makes high-throughput experimentation possible [9][10][11]. For single-cell research as an example, microfluidic systems have been developed to trap, culture, sort, image, and sequence single cells, and have begun to contribute to the biology in a meaningful way [12][13][14][15].

Here we will review how microfluidic tools have been used to gather both phenotypic and genotypic information at single-cell or individual organism level, with higher throughput and better precision than conventional methods (Figure 1). We also showcase some specific examples of how the advantages of microfluidics are exploited to gain insights into the biology (Figure 2).

Facilitating Measurements and Enhancing Precision of Perturbations

Microfluidics has been a touted tool for biology for two main reasons: similar length scales with biological systems (e.g. macromolecules, cells, small organisms), and unique flow characteristics and field properties at micro scale (e.g. laminar flow, enhanced surface effects). These microfluidic properties are conducive for handling and manipulating biological systems more precisely than conventional methods, and therefore can facilitate measurements that may have been difficult otherwise [16]. Microfluidic devices have been used to isolate, culture and image thousands of cells individually over an extended period of time for single-cell systems such as yeasts and bacteria. For example, Crane *et al.* developed a system to monitor up to 1,000 cells of budding yeasts for over 60 hours in a well-defined and controlled environment to study yeast aging [17]. Traditionally, to perform lifespan studies on individual yeast cells, researchers would have to monitor individual cells via microscopy and manually remove daughter buds using a micromanipulator every time the cells divide [18]. Because of this laborious process, the sample sizes are often too small to generate statistically meaningful results. Microfluidics in combination with automated microscopy, in contrast, allows lineages to be traced and the lifespan of large number of

individual yeast strains to be accurately measured [17][¹⁹]. These measurements could potentially facilitate understandings of functions of genes and networks in development and aging. In addition, the devices can also introduce precise environmental stimuli to perturb yeast cells at different life stages. Crane *et al.* were able to observe longitudinally how yeast cells respond to environmental stress as they age. Because yeast cells are non-adherent, it would have been very difficult to perform these experiments without microfluidic devices. It is worth noting that other trap and manipulation methods using hydrodynamics and electrowetting can be incorporated to manipulate cells and generate specific perturbations [20][²¹][²²]; these methods may prove useful under different experimental requirements (e.g. electrowetting being most useful for an open system where samples need to be frequently extracted or injected).

Another utility of microfluidics is the ability to provide standardized, precise, and highly reproducible stimulation to individual samples [²³][²⁴][²⁵]. In a recent example, Chingozha *et al.* designed a fluid exchange chip combined with cell trap array for non-adherent cells to study T cell response to varying chemical stimuli [²⁶]. In conventional platforms, protein or RNA analyses at the single-cell level such as immunochemistry and smFISH are difficult to perform due to uneven liquid exchange across all cells; furthermore, cell identities are lost between experimental manipulations. In contrast, the microfluidic design not only allows live imaging of a large number of cells in parallel, but also allows rapid, yet gentle fluid exchange to allow for such protein or RNA analyses to be done with precision. Preliminary studies using this type of setup demonstrate that in some instances, some early signaling may be a good predictor of ultimate functional output of the cell, but not universally and not always. Clearly this type of insight would have escaped through the analysis of average cell population behavior. One could imagine that the combination of microfluidic technology and genetic manipulation technologies, such as gene editing and optogenetics, can offer new ways to study and control more complex cellular or multi-cellular networks.

Increasing Throughput of Assays

Many system-wide studies mandate that high-throughput assays be used to gather large amount of data for large sample sizes. Parallel miniaturized reaction/culturing chambers or fast serial measurements can deliver the throughput of up to thousands of sample a day. However, for studies that requires even higher throughput, droplet microfluidics technology, which can analyze millions of samples in parallel, may offer a better solution [²⁷][¹⁰]. In microdroplets, each assay is compartmentalized in an aqueous media surrounded by an immiscible oil. The device can generate and manipulate droplets at kHz frequency to facilitate the screening and sorting of millions of cells or experimental conditions [28][²⁹]. Companies such as Raindance are already providing commercial droplet-based services such as droplet digital PCR to laboratories [³⁰]. In a particular application, Mazutis *et al.* conducted binding assay screen for detecting antibodies of over one million single mouse hybridoma cells in under 6 hours [³¹]. Over the last decade, many refinements of droplet techniques on-chip have led to simplification of operation of these devices, and an enlarged repertoire of applications. Some examples include systems for studying bacterial population dynamics [32], studying kinase signaling [33], performing directed evolution in yeasts [34] [³⁵], screening *C. elegans* [³⁶], and performing mammalian embryo vitrification [37].

Furthermore, parallel development of biologically compatible surfactant and oil systems have also contributed to the rapid growth of the field [38][39]. With these improvements, the technology has been adopted in many non-engineering labs for a variety of applications including high-throughput screening and sequencing (e.g. Drop-Seq).

Enabling Single-cell Functional Omics

Two exciting applications of microfluidic technologies for single-cell functional omics in the last few years are multiplexed transcriptome and proteome analysis for single cells [40][41]. Traditionally, whole transcriptomes or RNA-seq for profiling genetic expression has only been done on bulk tissues; yet, there is a growing demand to understand transcription dynamics at the single-cell level, as the large heterogeneity of genetic expression at single-cell level may be biologically significant [42]. In microfluidic systems, individual cells can be captured and lysed. The mRNA are then reverse-transcribed into cDNA inside the microfluidic channel by fixing the reverse transcription protein on chip. These cDNAs are then collected, and can either be sequenced for a subset of the genome (now commercialized by Fluidigm [43][44]) or pooled and sequenced off chip [45]. Whole transcriptome sequencing at single-cell resolution using these methods has already been performed on mouse bone-marrow-derived dendritic cells [43], identifying a small group of cells that can drive paracrine signaling in inflammatory responses. In another example on human brain cells [44], the experiments identified the heterogeneity of genetic expression at single-cell level and expanded the previously known cell types. Recently, development of Drop-seq allows highly parallel genome-wide expression profiling, further increasing the throughput of transcriptional analysis. Drop-seq works by encapsulating single-cells, lysing them, and then tagging cDNAs with unique oligonucleotide sequence for each cell. Tagging allows for sample pooling and high-throughput sequencing [46], and keeping each reaction in droplets can reduce amplification noise [47]. The results from both earlier and the recent Drop-seq studies suggest that conventional techniques of defining cell types based on gross phenotypes are vastly underestimating the diversity in many tissues, including the brain and the immune systems. As Drop-seq becomes routine in many labs, it is likely that we will see a surge in cell types identified and a better understanding their roles in functions and physiology.

In addition to advances in transcriptomics, microfluidics has also facilitated progress in single-cell proteomics. Traditionally, mass spectrometry is used to map the proteomes of tissue samples or cell culture samples; in general, a large number of cells have to be used, and the data reflect the “average” cell. Analyzing proteins with single-cell resolution remained challenging not only because of the limits of detection in mass spectrometry, but also because of the lack of the ability to handle the physical samples and the operations for sample prep. While microfluidics has not solved this problem thus far, strides have been made to miniaturize electrophoresis to handle single cells by, for example, performing single-cell western blotting on hundreds of cells concurrently [41][48]. The design of these microfluidic assays are highly modular; thus other chip components can also be integrated after the cell lysing step, should they be required. Future development in the omics assays are likely to be in increasing the capacity of proteomics analyses, which may be done by

pushing the limits of electrophoretic techniques or integrating with powerful mass spectrometry techniques.

Controlling and Analyzing Inter-cellular and Multi-organism Interactions

Often it is necessary to study complex systems consisting of multiple cells and cell types, or different organisms, e.g. microbial communities. Microfluidic system can be used to conduct a large number of well-controlled individual assays, by mimicking the *in vivo* environment for cells, as well as providing precise spatial and temporal microenvironment control [49]. Additionally, it is a useful tool to make co-culture systems to study interactions among individual cells (as in tissues) and among organisms (e.g. in ecosystem or in pathogen-and-host system). For example, Dura *et al.* designed a microfluidic device to manipulate pairs of cells into contact with a defined amount of time to study lymphocyte activation dynamics [50][51]. In another example, Austin and colleagues used microfluidic devices to study bacteria competition and evolution under different environments; these experiments elucidated how *E. coli* filamentation enables the bacteria to develop resistance to antibiotic exposure [52][53].

For multi-cellular systems, with the rapid development of 3D printing technology, more complicated microfluidic device with 3D scaffolds have been developed to accommodate the structural complexity in multi-cellular systems, such as in functional biological tissues and stem cell aggregates [54][55][56][57]. These functional tissues and organ-on-chip cultures can be then used to mimic normal or pathological physiology. Doing so on-chip has the advantage of the ability to control perfusion rate, dissolved oxygen, geometry and mechanical environment, fluid flow, etc. [58]. These systems can reduce the cost of drug testing; when patient-derived cells are used, they can be highly specific and personalized [59].

Furthermore, microfluidics has been a useful tool for studying systems neuroscience or physiology in model organisms such as *C. elegans* [60] and zebrafish [61]. Recent applications in *C. elegans* include approaches to understand how neural circuits give rise to behavior [62][63], to study neurodegeneration [64], to dissect how genes control neural developmental program, e.g. synaptogenesis [65], and to image whole-brain activities in different conditions [66]. In these examples, without microfluidics, it would have been difficult to manipulate the fluids and samples required to conduct the experiments. Other recent applications that demonstrate the high-throughput nature and the power of automation include studies of complex physiological responses such as the aging and stress response [67][68][69]. Here, the devices facilitated longitudinal gene-expression profiling under many perturbation conditions, which would have been difficult with conventional manual experimentation and measurements.

Conclusion and Perspective

Many systems biology problems involve 1) making system-wide measurements, 2) generating computational or conceptual network models, 3) creating testable hypotheses using network models, and 4) testing hypotheses by introducing controlled perturbations to

the system. As we have illustrated in many of the successful examples in this review, microfluidics, when properly designed, can enhance and aid many of these experimental and conceptual developments. We note that as design and fabrication become more standardized, simpler, and more economical, more laboratories are likely to adopt these experimental approaches. In combination with other tools, such as optogenetics, next generation sequencing, and 3D printing, microfluidics is likely to contribute to gaining more insights to complex biological systems that systems biology aims to understand.

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Highlights

- Microfluidics enables culturing, perturbing, and measuring cells and organisms precisely.
- Microfluidics can scale up and increase the throughput of biological experiments.
- Droplet microfluidics is particularly powerful for omics research.
- Microfluidics enables studies of complex intercellular and inter-organism interactions.

Figure 1.
Advantages of microfluidic technology and applications for systems and synthetic biology.

Figure 2.
Example and Advantages of Microfluidic Platform for Studying Biological Systems