

Chang Lu · Scott S. Verbridge *Editors*

Microfluidic Methods for Molecular Biology

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Preface

From the optical instruments that first provided a window into the microscopic world, to molecular analysis tools that have helped clarify the genetic underpinnings of life, the most significant advancements in the biological sciences over the past century have largely been driven by the availability of powerful new quantitative tools. A more recent theme in biotechnology has been the miniaturization of analytical tools, enabled in large part by adapting and developing the methods of the microelectronics industry. Microfluidics as a field has experienced a period of rapid development and evolution since the 1980s. Most of the early microfluidics work focused on miniaturization of tools used in analytical chemistry (e.g., chromatography and electrophoresis). Microfluidics provided unparalleled flexibility for miniaturization, integration, and automation. This permitted the creation of devices that were substantially more sophisticated than tools used in conventional analytical chemistry, and at a substantially lower cost of production and operation. Seminal early efforts of the field led to major innovations in both material science (e.g., the wide use of polydimethylsiloxane) and technology (e.g., two-layered pneumatic valves). These important developments greatly expanded the applications of microfluidics and have underpinned a more recent renaissance in microfluidics for biological applications.

With the explosion of genomics in the 1980s (and later of additional “omics” fields), molecular biology has always been an important area of application for miniaturized devices, given these devices’ unique access to the size scales relevant to the function of cells. Protein/nucleic acid separation and PCR-based analysis are widely practiced on microfluidic devices. Single-cell analysis has been an intensively explored direction in recent years, due to the unique size advantage associated with microfluidics for single-cell manipulation. With the decreasing cost of next-generation sequencing, recent years have witnessed substantial efforts directed towards genome-wide studies (as opposed to investigations focused on specific loci).

In parallel to new opportunities in basic science, there has been an increasing demand for well-established and robust microfluidic technologies that may have a

direct impact on clinical practice and therapies. Personalized Medicine (PM), more recently re-branded as Precision Medicine, provides just such a unique opportunity. PM is based on the premise that every patient is unique at the tissue, cellular, and molecular level. Thus conducting molecular biology tests on patient samples is essential for providing clinicians with genomics, transcriptomics, epigenomics, and proteomics information that will ultimately improve their ability to optimize decision making for individual patients. Microfluidics offers the ideal platforms for handling and analyzing low quantities of cell/molecular samples to enable this exciting personalized approach; however there remains much work to be done before this paradigm is routine practice.

This book volume highlights recent progress on the topic of microfluidics for molecular biology studies. We cover various aspects of current microfluidics research in this growing field, which now spans the disciplines of biology, physics, chemistry, forensics, engineering, earth and atmospheric sciences, and beyond. Chapters are presented on various types of molecular analysis (genetic, epigenetic, proteomic, and next-generation sequencing), use of model organisms and patient materials, analysis at bulk and single-cell levels, techniques on cell culture and isolation, and a variety of different platforms at the technological cutting edge of these respective fields (flow, droplet, and paper-based microfluidics). We are hopeful that new microfluidic tools will continue to enable new insights into basic science as well as technology and biomedicine. To analyze molecular populations at the resolution of single cells or even single molecules will undoubtedly provide new ways of understanding complex biological processes, for example the dynamics of the adaptive immune system, or the role of tumor heterogeneity in cancer. High-resolution tools translated to the clinic could enable entirely new ways to treat a patient's own disease, as opposed to treating a hypothetical average patient as is the current pharmaceutical paradigm. We hope to provide a succinct but comprehensive picture of the state of the art for microfluidic molecular assays, and we look forward to the advancements yet to come in this exciting and rapidly progressing field.

Blacksburg, VA, USA
December 15, 2015

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Chapter 1

Microfluidic Platforms for Quantitative Biology Studies in Model Organisms

Daniel A. Porto*, Tel M. Rouse*, Adriana San-Miguel*, and Hang Lu

Abstract The use of lab-on-chip tools has been adopted in a wide variety of scientific fields. Hundreds of applications that speed up, miniaturize, or enable otherwise unfeasible assays have emerged in the last couple of decades [1, 2]. The microfluidic toolbox offers several advantages which make it a very attractive resource for biological studies: reduced sample volume, control of spatiotemporal chemical compositions, streamlined assays, precise and predictable fluid flow regimes, portability, and integration with sensors, actuators, controllers, and automation systems [1–3]. The main drive of the field has thus far focused on the development of microfluidic tools that replace conventional methods with proof-of-principle applications. However, widespread adoption of these technologies for fundamental research is still in progress. Microfluidics has nonetheless had a significant impact in fundamental biological studies with model organisms [4, 5]. In this article, we provide an overview of the current state of the field, the impacts of microfluidics in model organism research, and the outlook, challenges, and opportunities for the future.

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1.1 Introduction

The use of lab-on-chip tools has been adopted in a wide variety of scientific fields. Hundreds of applications that speed up, miniaturize, or enable otherwise unfeasible assays have emerged in the last couple of decades [1, 2]. The microfluidic toolbox offers several advantages which make it a very attractive resource for biological studies: reduced sample volume, control of spatiotemporal chemical compositions, streamlined assays, precise and predictable fluid flow regimes, portability, and integration with sensors, actuators, controllers, and automation systems [1–3]. The main drive of the field has thus far focused on the development of microfluidic tools that replace conventional methods with proof-of-principle applications. However, widespread adoption of these technologies for fundamental research is still in progress. Microfluidics has nonetheless had a significant impact in fundamental biological studies with model organisms [4, 5]. In this article, we provide an overview of the current state of the field, the impacts of microfluidics in model organism research, and the outlook, challenges, and opportunities for the future.

Microfluidics takes advantage of the physics that govern fluids at small scales. The most important phenomena that dominate at the micro-scale are laminar flow and surface effects [6, 7] (Fig 1.1). Laminar flow entails a predictable stream behavior and minimal mixing. Manipulation of fluids at the micro-scale thus becomes precise and reproducible. These characteristics are useful to not only control spatiotemporal chemical compositions [8–12] but also to manipulate specimens via the exertion of forces driven by fluid flow [13]. Operating in a laminar flow regime implies that mixing of fluids requires special consideration, as this occurs solely through diffusion and Taylor dispersion [7]. Hence some assays require the incorporation of channel geometries that enable efficient fluid mixing within a desired residence time [14, 15]. Surface effects refer to the high surface-area-to-volume ratio of fluids constrained at the micro-scale [7, 16, 17]. These can be advantageous in the drawing of liquid via capillary forces that act through surface wetting [7, 17], or through field effects in electro-osmotic flows [16]. The high surface-to-volume ratio also facilitates interfacial transport and reactions [17]. At the same time, the small length scales enable fast changes in temperature and chemical composition. These, along with small sample volumes, are some of the main advantages that microfluidics offers. In particular, for model organisms such as bacteria, yeast, cells, nematodes, and some higher-order organism, the length scales of microfluidic channels are comparable to the specimen of interest (~ tens to hundreds of microns), providing the spatial resolution required for individual trapping, handling, culturing, and perturbation.

Early microfluidic devices were made mostly of silicon and glass, as these materials were ubiquitous in the semiconductor industry. Hard and brittle, silicon