Chandra K. Dixit · Ajeet Kaushik *Editors*

Microfluidics for Biologists

Fundamentals and Applications

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Preface

Microfluidics has revolutionized the way we deal with biological samples and biological matrix. It has enabled us to understand how a single cell is completely different from the information we can obtain by current tools and techniques. It is because of the microfluidic technology we are able to study physiology of a single cell and to understand the heterogeneity in the cellular population of the same descent. This is just one example of how microfluidics has changed the way we perceive biological information. This technology has enormous applications in every field of life sciences, from basics to industrial to diagnostics. However, there is a big communication gap between biologists and microtechnologists, which is due to a lack of training in the fields other than theirs.

In the first chapter of this book, we have presented the fundamentals of physics that govern microfluidics. These principles are presented in such a way that biologists can easily understand what controls the fluidics and how to use those for studying biological phenomenon. The second chapter of this book is dedicated to acquaint biologists with an overview of tools, techniques, and applications of microfluidics. The following chapters will cover manufacturing methods for developing custom microfluidic tools including 3D printing. Valving for controlling fluids in fluidic tools is also explained. Surfaces, sensors, and their integration are described such that the layman can understand the concepts. In the following chapters, the application of microfluidics in the field of cell and molecular biology, single cell biology, and disease diagnostics are introduced with simplicity. All these chapters are discussed in relation to commercial technologies so biologists can better correlate functioning of these tools with applications they desire to employ. This book is an attempt to describe the need of novel microtechnologies and their integration strategies for developing a new class of assay systems to retrieve the desired health information of patients in real time. This book also describes the selection and integration of sensor components and of operational parameters for developing point-of-care (POC). System-on-a-Chip (SoC), Diagnostic-on-a-Chip (DoC), and Lab-on-a-Chip (LOC) are the core to the next-generation bioanalytical sciences; therefore, this book can be lab assistance for those who work with biology-microfluidics interface, thus helping them to understand these systems and allowing them to make educated decisions on selecting the nature and type of microtechnologies that suits best to their methods thereby enhancing the rate of translational research in the field.

Salient Features of This Book

- This book serves as a resource guide for biologists and chemists to understand the complex physics of microfluidics.
- Describes the preparatory methods for developing 3-dimensional microfluidic structure and their use for LOC designing.
- Explains the significance of miniaturization and integration of sensing components to develop wearable sensors for POC.
- Demonstrates the application of microfluidics in life sciences and analytical chemistry including disease diagnostics and separations.
- Motivates new ideas related to novel platforms, valving technology, miniaturized transduction methods, and device integration to develop next-generation sequencing platforms, future diagnostic systems, and platforms for single cell biology applications.
- Discusses the future prospects and challenges of the field of microfluidics in the areas of life.

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Chapter 1
Fundamentals of Fluidics

Chandra K. Dixit

1 Introduction

Microfluidics has had tremendous impact on miniaturization of biological experiments by reducing the reagent volumes, shortening the reaction times, and enabling multiplexed parallel operations by integrating an entire laboratory protocol onto a single chip (i.e., lab-on-a-chip or LOC). Best examples of microfluidic tools in biology are Gene chips, Capillary electrophoresis, CD-based inertial cell separation devices, integrated transcriptome analysis systems, and others. Along with miniaturization comes a tremendous opening at the microscale where slight manipulation in physics can provide unprecedented number of applications for each design. An understanding of the physical processes at microscale and their dynamics can allow biologists to leverage those for performing experiments that are practically not feasible at macroscale. Since microfluidics can allow new processes and experimental paradigms to emerge therefore, here we will focus on fundamentals that predominantly govern the processes at microscales and how we can manipulate those to address problems in the field of biology.

2 Microfluidic Physics

Dimension is the key in understanding the magnitude of a physical event taking place. Prior to discussing physics of microfluidic processes we must first understand that on what we are working. Few important symbols representing physical quantities and the microfluidic scales that are mainly relevant to biologists are mentioned in Tables 1.1 and 1.2, respectively.

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Table 1.1 Common symbols for physical parameters

Greek letter symbols			
α	alpha	λ	lambda
β	beta	μ	mu
γ	gamma	ν	nu
Δ	delta	Π	pi
ε	epsilon	ρ	rho
ζ	zeta	σ	sigma
η	eta	τ	tau
Θ	theta	ω	omega
κ	kappa		

Table 1.2 Length scales for common biological moieties

Sample matrix	Approximate scales
Distance between molecules in a liquid	0.1 nm
Distance between molecules in a gas	3 nm
Mean free path between collision in a gas, air at ambient pressure (λ)	61 nm
Sample	
Protein, lipid molecule of the membrane	1 nm
Virus	10 nm
Cells	1–20 μm

These are few illustrative sample matrices and sample types that are routinely employed in biological analysis. Given the sizes, our focus should be on the phenomenon that can be used to manipulate micron and sub-micron entities. Reagent mixing, reagent delivery, cell capture, and shear-free conditions for biological analysis are few typical applications that are sought by biologists. We will understand physical entities in this chapter with respect to these applications that will allow developing an understanding of microfluidics.

2.1 *Hierarchy of Dimensions*

Before advancing to the complex physics dominating the micron regimen, we must first review the basic concepts and their respective dimensions. Table 1.3 summarizes few of the most basic scaling entities.

Entity	Dimension
Size	$[l]$
Surface	$[l]^2$
Volume	$[l]^3$
Van der Waals	$[d]^{-3 \text{ to } -7}$
Various Forces	$[l]^{1 \text{ to } 3}$
l is size of an object,	
d is distance between two objects	

Table 1.3 Scaling laws: variation at changing length scales

Quantity	Scaling law
Time	$[l]^0$
Length	$[l]^1$
Area	$[l]^2$
Volume	$[l]^3$
Velocity	$[l]^1$
Acceleration	$[l]^1$
Density	$[l]^{-3}$
Viscosity	$[l]^{-2}$
Diffusion time	$[l]^2$
Reynolds number	$[l]^2$
Peclet number	$[l]^2$
Hydraulic resistance	$[l]^{-4}$

With our previous knowledge of physical processes, we can realize that size, shape, and volume have tremendous impact on the forces acting upon/between bodies. For example, let us consider the force exerted upon a body by earth. This force is called gravitational pull and is represented as the ratio of the product of masses of earth and ours to the squared distance between us. As we realize this force has dimensional dependence on the distance between the two bodies, which is $[l]^2$. Similarly, a body flowing through a water stream will experience some force exerted upon it by the flow. This is dependent on the size and surface of the body and is somewhat close to how biomolecules and cells will feel in the microfluidic channels. Therefore, we must now look few years back in high school physics, which is actually the foundation to our advanced understanding of microfluidics.

2.2 *Non-dimensionalization and Dimensionless Numbers*

This section is intended to introduce the concept and importance of non-dimensionalization because you will now know terms that will be commonly used throughout the text; if it is hard to understand at this point then these can be revisited once all the basics are learnt. Dimensions are critical in physical analysis as they draw boundaries around a physical quantity by defining them in dimensions. Their importance becomes predominant when we are working at structures in micrometer range where surface area increases drastically relative to volume. This characteristic dependence of physical processes on dimensions must be addressed in such a way that the process can be explained as a function of the intrinsic properties of the fluid rather than the dimensions of those properties. In other words, we must make equations governing these processes without any resultant dimensions. This can be achieved by carefully replacing quantities in those equations with others, such that their dimensions cancel out each and have no net dimensional dependence. These quantities may be constants and can be employed for understanding the relative importance of entities within the process itself. Thus, non-dimensionalization is known as removal of units from the mathematical expression of a phenomenon by substituting with appropriate variables. This is also termed as *scaling*.

Scaling reduces the dependence of the process on several variables and significantly contributes to understand the relative importance of the physical quantities in the process and to realize the variation in their dimensions. This certainly helps in neglecting the smaller terms from the equation, which simplifies the associated physics. Therefore, it allows understanding physics at smaller scales and thus, is very important in microfluidics.

We will not deal scaling in great detail as it is a complex method but generally non-dimensionalization can be achieved via following steps:

(a) Identify the unit for which scaling is required; developing a scaling law
(b) Identify all the variables dependent and independent to that unit
(c) Identify a set of physically-relevant dimensionless groups and plug them in
(d) Determine the scaling exponent for each one, and
(e) Rewrite the equations in terms of new dimensionless quantities.

Such dimensionless numbers are crucial for exploring fundamentals of the physics governing microfluidics. The essential fluid physics of a system is dictated by a competition between various phenomena. This competition is expressed via a series of dimensionless numbers capturing their relative importance. These dimensionless numbers (Tables 1.4 and 1.5) form a sort of 'parameter space' for microfluidic physics.

2.3 Hydrostatics: Physics of the Stagnant

Fluids, liquids and gases, are defined as a material which will continue to deform with the application of a shear force. These are governed by certain basic rules of physics. Fluids have a special property to mention, they flow but only under the influence of external forces; these are mainly governed by **pressure, field gradients, surface tension, and gravity**. Since we will be mainly dealing with liquids therefore, our main focus is on the concepts of hydrostatic and hydrodynamic fluidics. As the name suggests hydrostatics and hydrodynamics are processes related to static and flowing liquids, respectively. Both these processes are controlled by associated physical parameters that we will discuss in this section.

Table 1.4 Dimensionless numbers in fluid mechanics

Dimensionless number	Details	Formula
Reynolds Number	Inertial force/Viscous force convective momentum/viscous momentum *Forced Convection*	$Re = \rho UL/\eta = UL/\nu$
Prandtl Number (heat) Prandtl-Schmidt Number (mass)	Momentum/Species diffusivity Used to determine fluid or heat or mass transfer boundary layer thickness	$Pr_{heat} = \nu/\alpha = \eta C_P/K$ $Pr_{mass} = Sc = \nu/D = \eta/\rho D$
Péclet Number (heat) Péclet Number (mass)	Convection transport rate/Diffusion transportation rate	$Pe_{heat} = RePr = UL/\alpha$ $\alpha = k/\rho C_P$ $Pe_{Mass} = RePr = UL/D$
Nusselt Number (heat) Nusselt-Sherwood Number (mass)	Length scale/Diffusion boundary layer thickness Used to determine the heat (h) or mass (h_D) transfer coefficient	$Nu = \left[f_\varepsilon Re(Pr)^{1/3}\right]/2$ $Nu_{heat} = hL/k_{fluid}$ $Nu_{Mass} = h_D L/D_{fluid}$ $L = A_s/Pm$
Grashof Number (heat) Grashof Number (mass)	Natural convection buoyancy force/ Viscous force Used to calculate Re for buoyant flow Controls the lengthscale to natural convection boundary layer thickness *Natural Convection*	$Gr_{heat} = g\beta(T_s - T_b)L^3/\nu^2$ $Gr_{Mass} = g\beta_C(C_{as} - C_{aa})L^3/\nu^2$ $\beta = -\left[(\partial\rho/\partial C_\alpha)_{T,P}\right]/\rho$
Rayleigh Number (heat) Rayleigh Number (mass)	Natural convection/Diffusive heat or mass transport Used to determine the transition to turbulence	$Ra_{heat} = GrPr = g\beta(\Delta T)L^3/\nu\alpha$ $Ra_{mass} = GrPr = g\beta_C(\Delta C)L^3/\nu D$
Knudsen Number (to analyze extent of continuum)	Slip length/Macroscopic length	$Kn = \beta/L$
Richardson Number	Buoyancy/Flow gradient	$Ri = g(\Delta\rho)/\rho U^2$
Eötvös (Eo) or Bond Number (Bo)	Body forces/Surface tension Used together with Morton Number to determine shape of drops or bubbles in surrounding fluid or continuous phase	$Eo = Bo = \left[(\Delta\rho)gL^3\right]/\sigma$
Capillary Number	Viscous forces/Interfacial forces	$Ca = \eta U/\sigma$
Elasticity Number	Elastic effects/Inertial effects	$El = \theta\eta/\rho R^2 = Wi/Re$
Weissenberg Number	Viscous forces/Elastic forces	$Wi = \gamma'.t_s$
Deborah Number	Stress relaxation time/Time of observation	t_s/t_o

Table 1.5 Common physical entities in fluid mechanics

Physical entity		Unit	Dimension
U	Characteristic velocity	m/s	LT^{-1}
L	Characteristic length	m	L
T	Temperature	K	Θ
T_s	Surface temperature	K	Θ
T_b	Temperature of the bulk	K	Θ
D	Mass diffusivity	m^2/s	L^2T
C_p	Specific heat	J/Kg.K	$L^2T^{-2\Theta-1}$
C_{as}	Concentration of species a at surface	Kg/m^3	ML^{-3}
C_{aa}	Concentration of species a in ambient medium	Kg/m^3	ML^{-3}
A_s	Surface area of the pipe	m^2	L^2
Pm	Perimeter	m	L
η	Dynamic viscosity	$Pa.s = Ns/m^2 = Kg/ms$	$ML^{-1}T^{-1}$
ν	Kinematic viscosity	m^2/s	L^2t^{-1}
σ	Surface/interfacial tension	$Kg/s^2 = N/m$	MT^{-2}
ρ	Density	Kg/m^3	ML^{-3}
β	Coefficient of thermal expansion	1/K	Θ^{-1}
α	Thermal diffusivity	m^2/s	L^2T^{-1}
k	Thermal conductivity	W/mK	$MLT^{-3}\Theta^{-1}$
h	Convective heat transfer coefficient	W/m^2K	$MT^{-3}\Theta^{-1}$
h_D	Convection mass transfer coefficient	m/s	LT^{-1}
λ	Mean free path	m	L
γ	Specific weight	N/m^3	$ML^{-2}T^{-2}$
R	Radius of the pipe	m	L
Θ	Stress evolution		
t_s	Stress relaxation time for the fluid	s	T
t_o	Time of observation of event	s	T
γ'	Sheer rate	1/s	T^{-1}

Pascal's Law

- Pressure applied anywhere to a fluid transmits the force equally in all directions
- Change in pressure disperses equally throughout the fluid
- Force acts at right angles to any surface in contact with the fluid
- Hydraulic press is the representative example

Hydrostatics is the physics of pressure confined within the definitions of Pascal's law and Archimedes principle constitute hydrostatics

2.3.1 Pressure and Pumping

Consider a cuboidal bottle filled with water to a height of one meter with length and width of the bottle at 5 cm each. The liquid in bottle is not continuous, instead a stack of several individual layers of water molecules, such that each layer is parallel to each other and continuously interacting with each other.

Now, **PRESSURE** is how much force is exerted on a given area and is expressed as

$$P = F/A \tag{1.1}$$

where,

P is pressure, F is force exerted, and A is the surface area on which force is exerted.

SI unit of pressure is atmosphere (atm) and is equivalent to 10^5 Pascals, another unit for pressure and have dimension Nm^{-2}.

By the virtue of the definition of pressure, the top layer of the water molecules must exert a force on the layers beneath it over the surface area of the layer. Similarly, the top layer will do so on the last layer at the bottom. It is crucial to understand that for fluids under gravity, based on (1.1), pressure exerted by an upper layer on the one underneath is directly dependent on the distance between those layers expressed as height. From Fig. 1.1a, the pressure exerted by the liquid on the bottom of the container should be calculated as

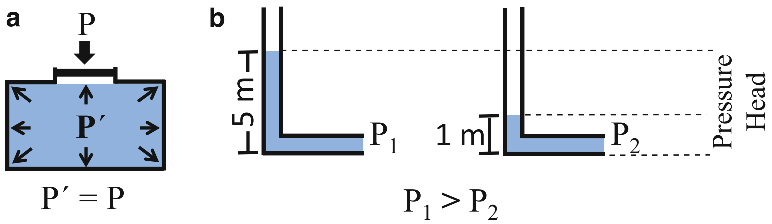

Fig. 1.1 Illustration of Pascal's law. (**a**) Pressure exerted at any point on a continuous fluid is dissipated equally in all directions on that fluid. This concept makes the basis of hydraulic press and brakes. (**b**) An extension of Pascal's law is pressure head driven flows where the height of the liquid exerts a pressure on the lower layers. This concept of height-dependent pressure is used in pumping in microfluidics. As depicted, 5 m head will exert more pressure than 1 m head

$$F = mg \tag{1.2}$$

where,

m is mass of the liquid, and g is gravity constant.

Since,

$$m = \rho V \tag{1.3}$$

where,

V is volume of container, and ρ is mass density of the liquid.

Therefore, replacing (1.3) in (1.2) will give us

$$F = V(\rho g) = hA(\rho g) \tag{1.4}$$

such that volume = height of the liquid (h) * area of the surface (A = length * width)

Similarly, replacing (1.4) in (1.1) will give us the relation of height to the pressure

$$P = hA\rho g/A = h\rho g \tag{1.5}$$

Continuing with the case that we were discussing, in Fig. 1.1b pressure exerted by a layer on the other separated by certain height within the liquid will be

$$P_2 - P_l = \mathbf{\Delta P} = (h - h_l)\rho g = \Delta\mathbf{h}.\rho\mathbf{g} \tag{1.6}$$

Equation (1.6) constitutes the basic of **hydrostatic pressure-based pumping** in **microfluidic systems**. '**ΔP**' is known as pressure head.

2.3.2 Buoyancy and the Problem of Microfluidic Mixing

Buoyancy is the apparent loss of weight of a body when submerged in liquid and this is mainly known as Archimedes Principle. This loss is attributed to the resistance offered by the liquid to the body. Buoyancy from Fig. 1.2 can be mathematically expressed as

$$F_{net} = F_B(\text{buoyant force}) - F_g(\text{weight}) \tag{1.7}$$

$$= (\rho_f V_f - \rho_o V_o)\, g \tag{1.8}$$