PREFACE

This book aims to provide an overview of current exciting research topics in ultrasensitive bioanalysis; to provide an in-depth understanding of objectives, motivations, and future directions of the new frontiers of ultrasensitive bioanalytical research; and to introduce new ideas, new technologies, and new applications of ultrasensitive bioanalysis to a wide spectrum of research communities, including biological, biomedical, clinical, chemical, environmental, and materials science. The book strives to provide sufficient fundamentals and research background for readers to learn and apply these tools. The book is also structured in a way that can serve as a textbook or a primary reference book for an advanced analytical chemistry course or a special topic course for graduate and senior undergraduate students. I discovered the difficulty of finding effective teaching materials and suitable reference books for my students and collaborators while I taught the course and engaged joint research projects with colleagues in the department of biological sciences and engineering.

Ultrasensitive detection plays a vital role in advancing analytical chemistry and has been a primary driving force for the development of new analytical instrumentation and methodology. As analysis of biological samples (e.g., genomics, proteomics) and living systems (e.g., cellular and subcellular function) becomes more demanding, new platforms of ultrasensitive analysis using multiplexing, single nanoparticle sensing, nano-fluidics, and single-molecule detection have emerged and have become indispensable tools to advance biological, biomedical, and biomaterials sciences and engineering. Furthermore, the emerging research field of nanoscience and nanotechnology provides new possibilities for development of new analytical tools and instrumentation for bioanalysis. For example, the unique properties of nanoparticles offer enormous opportunities to develop new probes for real-time *in vivo* and *in vitro* imaging and sensing of individual biomolecules with sub-100-nm spatial resolution and millisecond to nanosecond time resolution. Such powerful capabilities will lead to the development of new analytical techniques to improve disease diagnosis and treatment, as well as to advance our fundamental understanding of important phenomena such as membrane transport, gene expression, enzyme activities, and intracellular and intercellular signaling. It is undeniable that analytical chemistry has evolved to be a vital player in all cutting-edge scientific research fields.

In this book, a diverse group of analytical chemists working in the forefront of ultrasensitive bioanalysis share their insights, visions, and latest results. The book is structured to offer appreciable background on the fundamentals and to provide brief overviews of current status of ultrasensitive bioanalysis. More importantly, this book focuses on showcasing the exciting opportunities of the new frontiers of ultrasensitive bioanalysis. Thus, this book is much more for the future than for the summary of the past and present.

This book includes 13 chapters and starts, in Chapter 1, by addressing the most fundamental question of single-molecule detection (SMD): How many measurements of a single

molecule are required to obtain representative molecular properties of ensemble distributions? It describes the advantages of SMD and its applications to virtual sorting and DNA fingerprinting of single genomes. Chapter 2 focuses on presenting a representative application of SMD to directly observing gene expression in living cells, illustrating potential applications of SMD for better understanding of living cellular mechanism and function. Chapter 3 offers an overview of SMD using fluorescence microscopy and spectroscopy, and it gives an example of studying membrane transport of living cells using single fluorophor molecules and single-nanoparticle optics to illustrate the distinguished advantages of SMD and non-photobleaching noble metal nanoparticles for probing living cellular mechanism and function. This chapter aims to introduce the basic detection schemes and experimental configurations of SMD and single-nanoparticle optics for bioanalysis and single-living-cell imaging.

Chapter 4 is devoted to the recent development of multifunctional nanoparticle probes, especially quantum dots (QDs), for ultrasensitive analysis of biomarkers in living cells and *in vivo* imaging, providing the latest advances and future possibility of this exciting research arena. To develop nanoparticle probes for living-cell imaging, smart drug delivery, and *in vivo* diagnosis, the primary challenge is to rationally design the functional nanoparticle surfaces that can specifically recognize biomolecules of interest and be biocompatible to living systems. Thus, Chapter 5 offers examples of the latest advances in designing surface functionality of nanoparticles that can specifically recognize protein and DNA surfaces. This chapter presents an overview of this exciting research topic and provides a wealth of experimental details and new insights into the future research direction.

Miniaturization and multiplexing have become important technology platforms in ultrasensitive analysis of biomolecules and profiling contents of individual living cells. Chapters 6 through 8 present several interesting research projects, reporting the recent development of this particularly exciting research field. Chapter 6 describes the new platform of using the state-of-the-art research tools, such as a laser-based system and droplet-based microfluidics, to selectively extract organelles from living cells for subsequent analysis. Chapter 7 presents the development of near-IR fluorescent dyes, time-resolved fluorescence spectroscopy, and multiplexing approach for DNA sequencing, offering more sensitive and informative genomic measurements. In contrast, Chapter 8 describes a new approach of directly determining genomic DNA samples with no need of labeling or PCR amplification. In this approach, surface plasmon resonance imaging (SPRI) is used as a label-free detection means, while nucleic acid microarrays serve as a multiplexing platform, which is coupled with surface enzymatic method to amplify SPRI imaging signal.

Like proteins and DNA, metal ions and small molecules play a key role in cellular function. However, quantitative and qualitative analysis of small molecules, especially metal ions, in living cells in real time remains extremely challenging. Chapter 9 gives a glimpse of research activities and emerging approaches in this extremely important research area and offers new prospects of the future research possibility and direction.

Chapter 10 describes the development and application of electrochemical methods to monitor the release of neurotransmitters in real time for better understanding communication among individual nerve cells, demonstrating the unique and powerful detection capability of electrochemistry. Chapter 11 introduces electrochemiluminescence (ECL) detection for ultrasensitive bioanalysis, describes its latest development and applications, and presents the potential challenges and exciting future research opportunities of ECL detection in ultrasensitive bioanalysis.

Mass spectrometry offers an unparalleled detection capability for identifying unknown analytes (e.g., tumor markers) and has demonstrated the possibility of profiling the chemical compositions of individual living cells. However, mass spectrometry currently provides very limited detection sensitivity. Chapter 12 illustrates the latest research development of using state-of-the-art mass spectrometry for single-cell analysis, highlights several representative examples, and presents the unique research challenges and opportunities in this exciting emerging new research field. Finally, the book is concluded with Chapter 13: it summarizes current cutting-edge ultrasensitive detection means in bioanalysis, along with their unique features and potential applications. This final chapter also offers a brief outlook on ultrasensitive bioanalysis in terms of emerging technologies and methodologies, along with new challenges and opportunities.

I hope that this book will serve as a valuable tool for beginning and well-established scientists, especially graduate students and the researchers who consider learning about and using ultrasensitive detection tools. I have selected one example of particular detection techniques and research approaches to illustrate the concepts, ideas, experimental design, and potential applications of research field, rather than providing a comprehensive review of an entire research field. It is hoped that through these selected examples, the readers will feel the vibrant and excitement of entire research field and will pursue so many other distinguished research works that are unable to be included in this book. Thus, I hope that I will have the forgiveness and understanding of my colleagues whose research work has not been presented here.

I am very grateful to all contributors for their enthusiastic support and for taking precious time from their demanding busy schedule to prepare the chapters. I would like to acknowledge each and every one of them for their generous effort and inputs. I wish to extend my gratitude to James Winefordner at University of Florida for his persistent invitations over years, as well as to Heather Bergman at Wiley-Interscience for her valuable assistance.

It has been a very pleasant and valuable experience for me to work on this book. I gained the first-hand appreciation and better understanding of the effort, time, energy, and persistence that are essential for an editor of a book. With an already saturated daily schedule of young academics, I had to devote all my weekends and holidays this past year to working on this book in the hope that it would reach readers in time to meet the demands of this very rapidly expanding research arena. I will be extremely delightful if readers, especially students and new investigators, find that the book stimulates their fascination in ultrasenstive bioanalysis and helps them to steer toward new research directions that ultimately lead to new scientific discoveries.

Despite my best effort, I am mindful that I could have done more in editing this book. Therefore, I am eager to hear the constructive comments and suggestions of my colleagues and readers. I am determined to continue to engage in vigorous research and teaching activities in this thriving research field, and I plan to prepare follow-up volumes in the coming years.

It is fitting to take this opportunity to acknowledge several funding agencies for their generous support of my research program: NIH (R21 RR15057; R01 GM0764401), NSF (BES 0507036; DMR 0420304), DoD-MURI (AFOSR #F49620-02-1-0320), DOE (DE-FG02-03ER63646), and Old Dominion University. This financial support allows me to actively participate in this exciting cutting-edge research field, acquire the first-hand research experience, widely interact with colleagues in the field, closely follow the advance of

research field, and gain in-depth understanding of the research direction, which are essential to the successful construction of this book.

Finally, I would like to thank my family, especially my parents, for their tireless support of this endeavor. Without their patience, love, and guidance, this mission would have been impossible.

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