Near-Infrared Applications in Biotechnology

edited by Ramesh Raghavachari
Near-Infrared Applications in Biotechnology
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**ADDITIONAL VOLUMES IN PREPARATION**

Knowledge is the true organ of sight, not the eyes.

_Panchatantra_

In loving memory of my father,

_who always had simple solutions for complex problems._
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Foreword

I truly enjoyed reading about the various applications described in this volume. Having worked in near-infrared (NIR) applications for pharmaceuticals for twenty years, I have looked forward to the day a reference book on NIR would be published in a field something other than food, textiles, or polymers. This collection of applications to biological systems is groundbreaking, to say the least.

The book includes chapters focusing on fluorescence: fluorescence in biological applications, the dyes themselves, immunoassays, fluorescence lifetimes, and DNA sequencing. These constitute the best compilation of NIR-fluorescence references I have seen. For biological applications, fluorescence spectroscopy may well prove to be of greater import than absorption methods. The chapters on medicine and single-molecule detection are unique in their completeness. Even the polymer chapter looks at the subject in a manner I have never seen. I compliment the editor on his choice of contributors and subjects.

One of the problems with NIR being applied to sophisticated samples has nothing to do with the physics or software involved; it's where the technique originated. It may be passé to imply that a person was born on the "wrong side of the tracks," but NIR has suffered greatly for its ancestry. The majority of "reputable" or "proper" techniques—mass spectrometry, nuclear magnetic resonance (NMR or MRI), electrophoresis, etc.—used in laboratory analyses are of "good breeding." They were discovered at distinguished universities, developed by "real" instrument companies, then introduced to industry for application. Not so for NIR.

Ah, poor little NIR! It was developed (as an analytical technique) at the U.S. Department of Agriculture, Beltsville, Maryland (largely by Karl Norris,
a nonacademic!) for wheat, soy, and other such "trivial" substances. Several small companies were started (mostly in Maryland) for the sole purpose of building NIR analyzers. Since the pedigree wasn’t there, NIR wasn’t taken seriously by the scientific world, in general. At NIR meetings through the 1980s, a researcher in cereals might point to a portion of the spectrum and state, “This is the protein peak.” Real spectroscopists would never refer to the combination band resulting from the carbonyl stretch and amide N-H bend in such a manner.

However, as Karl and “pioneers” such as Fred McClure, Phil Williams, “Woody” Barton, and others began explaining the science behind NIR, it began to be taken seriously. Textbooks by Williams and Norris (1), Osborne and Fearn (2), and Burns and Ciurczak (3) helped codify the science. The International Council for NIR now holds conferences around the world and Tony Davies publishes NIR News and the Journal of NIR. Conferences such as the Pittsburgh Conference and the Eastern Analytical Symposium have presentations for achievements in the near infrared. Both conferences showcase numerous workshops, short courses, and oral and poster sessions.

Yet, despite this recent wealth of legitimacy, the “art” of NIR remains a minor force in medicine and industry. It is still not a common part of any curriculum in any college. Indeed, even with the vastly different approaches of technique and instrumentation, it is still lumped with mid- and far infrared as “part of the infrared (IR) region of the spectrum.” Of course, it doesn’t help that practitioners of the art can’t even agree on the boundaries of the NIR spectrum. Within this very book, you will find several differing opinions as to this range.

Since NIR is generally agreed to have its genesis in the midrange IR, I have always considered the upper boundary at 2500 nm or 4000 cm⁻¹. This is where the combination bands of the C-H, N-H, and O-H vibrational modes begin. It is also the upper limit of lead sulfide (PbS), the most common detector used in NIR instruments. Therefore, the question of whether this limit should be 2500 nm, 3000 nm, or 3200 nm is moot. The majority of NIR instruments don’t go above 2500 nm.

The definition of the lower limit is the cause of the most fireworks. Silicon (Si) is the detector of choice for the lower end of the NIR; it is also popular for the visible and ultraviolet ranges, too. Thus, the division between NIR and visible becomes blurred. There are absorbances as low as the 700 nm range that can be considered higher overtones of vibrational modes in the IR. Indeed, transuranium elements have easily excited f-electrons that can be excited by NIR radiation. Thus, there are electronic transitions above 800 nm—clearly in the “true” NIR region—blurring the distinctions at this lower boundary.

As you read the contributions herein, you will recognize the variety of algorithms used to analyze the data. This is because the spectral data obtained, especially in complex samples such as bodily fluids or skin, is rarely clear-cut. The spectra in NIR seem to disobey all the rules of Beer’s law: the analyte is
often the smallest contributor to the spectrum, there is usually a strong interaction between analyte and matrix, there are no isolated peaks, and any single wavelength seldom gives a linear response to the analyte absorbance. We are forced to use chemometrics (see Chapter 11), that is, sophisticated mathematics, unlike typical UV or visible applications. The actual choices of sample sites, number of patients, algorithms, etc., seem almost subjective in NIR analyses.

With many choices (all leading to usable equations) available to the researchers and no clear paths to follow, the dizzying array of approaches is, to an outside observer or neophyte, confusing. This is how gas chromatography began; dozens of researchers making their own coatings, packing columns, and setting their own standards. In time, we had harmonization and uniform packing practices. Indeed, clearer and clearer guidelines are emerging for NIR analyses. In pharmaceuticals, Ritchie (4) has been proposing guidelines that combine American Society for Testing and Materials (ASTM), International Conference on Harmonization (ICH), and current Good Manufacturing Practices (cGMP) recommendations and guidelines for spectroscopic methods development.

With work such as that contained in this text, Near-Infrared Spectroscopy is destined to become an important tool in medicine. I foresee nonintrusive diagnostics becoming a reality in the very near future. I also see many current analytical methods being replaced by in-process spectrometric monitors. There is a bright future for NIR in the health services sector as this text demonstrates.

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Preface

The advent of modern technology has made the impossible possible, allowing a serious view of what the unaided human eye cannot see. I refer to the near-infrared region, defined by Sir William Herschel as “beyond the red” in 1800. The NIR region was first put to use by Karl Norris of the U.S. Department of Agriculture in the mid-1960s, for the spectral analysis of many substances using absorption in this region. In a similar manner, although many dyes have been discovered over the centuries, the ones fluorescing in the NIR region have become important only within the past two decades. The late bloom of this field of science can be attributed to nonavailability of economical diode lasers at these wavelengths (excitation source), silicon avalanche or lead sulfide solid state detectors, and advanced information technology to analyze the generated data.

Biotechnology is a confluence of biology, chemistry, physics, engineering, and information technology. Deciphering important biological information using biotechnology has led to many advances in medicine and genetics, and in general has contributed to the welfare of humanity. The combinatorial approach to drug discovery, diagnostics of diseases in its early stages, advances in genetic identity, gene mapping, and genomic medicine is truly moving to the forefront of biotechnology in developing prevention and cures for complex diseases. Cloning and genetic engineering of animals and plants, when put to proper use, will have great impact on medicine and will contribute to the overall well-being of humanity.

This book attempts to give a glimpse of the potential applications of this young field of science. The book is divided into two parts: NIR applications based on fluorescence and NIR applications based on absorption spectroscopy.
Both areas are covered from fundamentals to applications in several growing fields. In addition, there are answers to many important fundamental questions a novice might have.

The controversy regarding where the near infrared starts and ends is quite visible in this book. Each author has expressed his or her opinion without any editorial changes. As an editor, I have taken a stand to define this region in the introduction and have attempted a plausible explanation. The reader should be aware of this.

The first two chapters in Part A focus mainly on the aspects of fluorescence and reference to biological processes and the dyes fluorescing in the NIR region. These two chapters are an introduction to the basics of this developing field, giving some of the finer points, which many advanced books do not cover or adequately explain. The chapter on fundamentals of fluorescence is also tuned more toward a biological application, since there are innumerable books that cover the basics elaborately. The first two chapters are very relevant for many applications. Each application is thoroughly explained in the following chapters with many examples. Applications in DNA sequencing, bioanalytical immunoassays and medicine are obviously relevant to biotechnology. It may appear that the chapters on single-molecule detection and applications using fluorescence lifetimes are rather theoretical; on the contrary, one can see many biotechnology applications in this field that can evolve and become more prominent in the future.

One can see that there are not many applications in polymers. However, relevant data has set a foundation for progress in that direction. More recently, the possibility of a polymer-based near-infrared diode laser has become imminent (1). The most exciting chapter for any chemist is Chapter 10, “Beyond Biotechnology and into Popular Technology.” In the 21st century this will become very relevant to biotechnology as well. One can envision impregnation of near-infrared sensors into the chips that will provide vision for the blind and even expansion of their vision in the near-infrared region.

This is a science in its growing stages—as one article calls it, a “teen-age science.” The resurgence in this field of science is felt in all application areas as envisaged by any other fluorescence and absorbance application. Fluorescence Spectroscopy: New Methods and Applications, edited by Otto Wolfbeis (2) gives an idea of the possible applications that can emerge from the field of visible fluorescence. One day it may be a reality that all those applications would extend into the near-infrared region as well.

The second part of this volume projects the possible applications based on near-infrared absorption spectrometry. The first chapter in Part B describes the fundamentals of this science and tells the reader what to expect. The next two chapters cover the applications in medicine and pharmaceuticals, that are the most relevant to biotechnology. The chapter on biomedical applications of NIR
spectroscopy deals with the diagnostic aspects of medicine. Simplifying and making diagnostics of some diseases using blood, urine, and other noninvasive techniques would make diagnostics easier as well as saving time and money. The chapter on pharmaceutical applications is an abridged version of the forthcoming book edited by Ciurczak and Drennen (3). This comprehensive chapter explains applications starting from raw materials, in-process applications, and the finished product with several examples. Chapters 11, 12, and 13 were added to make this book a comprehensive text so rich in NIR studies that each topic by itself could have been made into a separate book. There are many textbooks that are dedicated to the science of NIR spectrometry and they are well referenced in these chapters.

The aim of this book is to give the reader an overview of this young field of science and provide the possibilities of further exploration. This text should give a head start in keeping up with the current research.

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A project like this involves many people without whom this task would have been a very difficult one. First and foremost, I would like to thank the contributors for their contributions and their great enthusiasm in spite of their very busy schedules. I would also like to thank Dr. Vish Bhadri of Amersham-Pharmacia Biotech, Dr. Dan Simpson, and Dr. Patricia Fulmer of Promega Corporation, and Dr. Judy Schanke of Epicentre Technologies for their comments and opinions on some of the chapters in this book. Especially in an edited volume like this, it is extremely difficult to coordinate every aspect of the book from all over the world. I thank the staff members of Marcel Dekker, Inc. who were involved with this project. Last but not least I thank my family for putting up with me during this time.

Ramesh Raghavachari

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