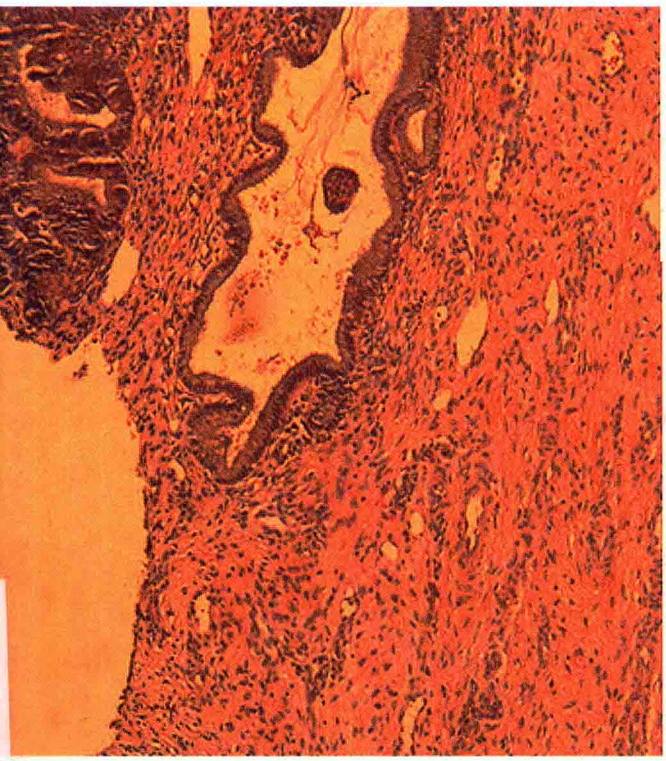
Modern Vibrational Spectroscopy and Micro-Spectroscopy

Theory, Instrumentation and Biomedical Applications

Max Diem



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Modern Vibrational Spectroscopy and Micro-Spectroscopy

Theory, Instrumentation and Biomedical Applications

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Modern Vibrational Spectroscopy and Micro-Spectroscopy

I would like to dedicate this book to my wife, Mary Jo, who put up with my hiding away in my home office for many an evening and my absentmindedness for 16 months during which this book was written.

I also would like to acknowledge the excellent and enthusiastic crew of post-docs, graduate and undergraduate students in my research laboratory at Northeastern University who was responsible for the majority of the work presented here (listed chronologically).

Miloš, Christian, Susie, Melissa, Brian, Tatjana, Jen (I), Ben, Ellen, Kostas, Antonella, Evgenia, Erin, Jen (II), Christina, Kathleen, and Doug.

Max Diem

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Preface

Although this book was conceived as a second edition of *Introduction to Modern Vibrational Spectroscopy* published by the author in 1993, it really is not a second edition, but a completely rewritten monograph on a subject that has changed so much in 20 years that the old edition appears seriously antiquated. In fact, few other areas of spectroscopy have undergone such radical changes in the past two decades as vibrational spectroscopy has: subjects that then were cutting edge technology have become so common that they have been part of undergraduate physical chemistry core laboratories for quite some time, and areas that were not even thought about 20 years ago are on the verge of commercialization. This enormous progress was spawned by a fortuitous co-incidence of many factors, such as instrumental advances that allow the collection of 10 000 infrared spectra within a few minutes, or the collection of Raman spectra of samples in the picogram regime in a few hundred milliseconds. The ready availability of pico- and femtosecond tunable laser sources has made routine acquisition possible of several nonlinear spectroscopic effects, based on excitation of short-lived vibrational states. Last not least, the enormous increase in computational power over the past 20 years has opened entirely new avenues for data processing and statistical analyses of the plethora of data collected in short times. In fact, the increased computational power is certainly one major enabling factor of an area of vibrational spectroscopic imaging, in which between 10 000 and 100 000 individual pixel spectra are collected through specialized microscopes and converted to pseudo-color images based on the vibrational spectroscopic features. This increase in computational power can easily be felt considering that in 1993, a top-end personal computer (PC) incorporated less than 1 MB of RAM, and was still based on Intel 386/387 processors and much of the scientific programming was carried out using FORTRAN compilers. Twenty years later, workstations with between 16 and 48 GB of RAM and multicore Pentium type processors are routine and available for a similar dollar amount required for the purchase of top-end PCs in 1993. The accessibility of thousands of routines for data processing and analysis, and being contained in the MATLAB environment, have offered sophisticated statistical and analysis routines even to the non-expert. Finally, theoretical developments have solved some puzzling aspects of vibrational spectroscopy; the prime example here is the understanding of the theoretical foundations of surface-enhanced Raman spectroscopy (SERS). In 1993, an overall agreement on the theoretical foundations of this effect had not been reached. Furthermore, the field seemed to be plagued by low reproducibility, which disappeared once the theory of surface enhancement was properly developed.

The need for inclusion of new techniques in vibrational spectroscopy, as well as the shifting research interests of the author, contributed to the very new format of this book, and the material contained herein. Furthermore, the strategies for teaching concepts of vibrational spectroscopy have changed both at the graduate and the undergraduate levels. Finally, the intended readership of this book has changed since the first edition addressed an audience of first year graduate students in chemistry while the present edition addresses, in addition, a readership interested primarily in the medical imaging and diagnostics fields. For these readers, the theory sections have been streamlined, and imaging aspects have been added. The first edition still contained a detailed discussion on how to perform empirical calculation of molecular force fields and vibrational frequencies. This subject has gone the path of dinosaurs, since such calculations are now all performed at the *ab initio* molecular orbital level. In addition, methods of data handling and analysis have been added that are necessary for both students and researchers in modern vibrational spectroscopy.

All these changes have forced a total rewriting of the book. Part I of the reworked monograph contains the theory of vibrational spectroscopy, presented both from the view of classical mechanics as well as from quantum mechanics. Although the later parts of the book deal mostly with large biological molecules, a short review of the group theoretical foundations of vibrational spectra of small molecules is included, as well as basic instrumental aspects. New techniques – surface and nanostructure-based spectroscopies, nonlinear effects, and time-resolved methods – are introduced as well. Thus, Part I represents a short course into "Modern Vibrational Spectroscopy" and is somewhat comparable to the first edition.

Part II deals with biophysical, medical, and diagnostic applications of vibrational spectroscopy. It starts with a review of the biophysical applications of macroscopic vibrational spectroscopy, a field that has produced ten thousands of papers on biomolecular structure, dynamics, and interactions. Subsequently, vibrational microspectroscopy (also referred to as vibrational microscopy) will be introduced. Although infrared and Raman microspectroscopy were certainly known and applied in 1993, their relevance was relatively low, and they were not included in the first edition. In contrast, both techniques are so prevalent now that they contribute to about 30% of sales in Raman and infrared spectral instrumentation. Both these techniques present their own challenges in instrumentation, data manipulation, and analysis, and are discussed in detail.

Vibrational microspectroscopy allows the detection and analysis of individual bacterial cells. In 1993, a few brave souls had embarked into this field and found that infrared spectra proved phenomenally sensitive in distinguishing different bacterial species [1]. The analysis of cells and tissue has undergone an explosive expansion during the past decade, and is now on the verge of becoming a major diagnostic and prognostic tool. Specialized journals, such as *Biospectroscopy* (now part of Biopolymers) and the *Journal of Biophotonics* are devoted to the application of (mostly) vibrational spectroscopy toward biological sciences and medicine. Many journals that used to concentrate on classical analytical chemistry have devoted entire issues to the emerging field of spectral diagnostics (see, for example, *Analyst*, Volume 135 and *J. Biophotonics*, Volumes 3 and 6).

Thus, the author hopes that this book will provide a detailed background, from the quantum mechanical foundation to the specific applications, for researchers in, or entering, the exciting and re-emerging field of vibrational spectroscopy.

In the years since the first edition was published, several new books have appeared discussing in detail several aspects of vibrational spectroscopy, such as D.A. Long's book on Raman theory [2], L.A. Nafie's book on Vibrational Optical Activity [3], volumes on Quantum Mechanics or Group Theory [4], monographs on microspectroscopy, and many more. The present book can impossibly compete with these specialized books on the details presented, since it still was conceived as an "Introduction" to modern vibrational spectroscopy. Therefore, the subjects discussed here are more appropriate for a researcher entering this field, or for advanced undergraduate students in Chemistry or the Life Sciences.

Finally, the author herewith apologizes categorically for one aspect in this book that is presented inconsistently throughout the chapters: the presentation of spectra from left to right and from right to left. Historically, Raman spectra have been presented mostly from "left to right," that is, from low to high wavenumber. Infrared spectra were originally presented from high wavenumber to low wavenumber, or from "right to left." Of course, this was due to the fact that the wavelength increases from "left to right" in this presentation. Although there are some recommendations by IUPAC on the representation of spectra – Raman spectra from "left to right" and infrared spectra from "right to left" – too many researchers have not abided by this rule; consequently, spectra are displayed in the literature both ways. Since many figures in this book are taken from the work of many researchers, these figures could not be reversed to a standard theme. Thus, the reader is reminded to pay particular attention to the direction of the wavenumber scale in the figures.

Boston, July 2014

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3. Nafie, L.A. (2011) *Vibrational Optical Activity: Principles and Applications*, John Wiley & Sons, Ltd, Chichester.
4. Cotton, F.A. (1990) *Chemical Applications of Group Theory*, 3rd edn, John Wiley & Sons, Inc., New York.

Preface to *Introduction to Modern Vibrational Spectroscopy* (1994)

The aim of this book is to provide a text for a course in modern vibrational spectroscopy. The course is intended for advanced undergraduate students, who have had an introductory course in Quantum Chemistry and have been exposed to group theoretical concepts in an inorganic chemistry course, or for graduate students who have passed a graduate level course in Quantum Chemistry.

There are probably a dozen or so recent books in vibrational spectroscopy, and a few classical texts over three decades old. This large number seems to discourage any efforts to produce yet another book on the subject, unless one is willing to pursue a novel approach in presenting the material. This is, of course, exactly what was attempted with the present book, and the approach taken will be outlined in the following paragraphs.

There are two classical and comprehensive texts on vibrational spectroscopy, *Molecular Spectra and Molecular Structure. II. Infrared and Raman Spectra of Polyatomic Molecules* by Herzberg [1] and *Molecular Vibrations* by Wilson *et al.* [2]. Both of these books are absolutely essential for an in-depth understanding of vibrational spectroscopy, and devote hundreds of pages to theoretical derivations. However, due to the rapid progress in instrumental techniques and computational methods and due to the fact that thousands of molecules have been studied since these two books were written, the practical aspects of these books are certainly limited. However, the value of these classic books for the serious vibrational spectroscopist is immeasurable, since they provide many of the fine points needed for a detailed understanding of the subject.

Among the more recent books, the reader will find either very specialized works dealing with one or a few specific topics of vibrational spectroscopy, or books that are more a compilation of data than a comprehensive text. The more practically oriented books often emphasize correlations of observed spectra with molecular structural features, and may contain large compilations of spectra and group frequencies, and only cursory treatment of theoretical principles. These books are essential for researchers who wish to employ vibrational spectroscopy as a qualitative structural tool.

However, neither of these could be used as a text book in a course, nor could they be used by a researcher who wants to gain insight into modern aspects of vibrational spectroscopy. Thus, the author was faced with the challenging task of writing a text that incorporates some theoretical background material which is necessary for the understanding of the principles of vibrational spectroscopy, in addition to computational methods, instrumental aspects, novel developments in vibrational spectroscopy, and a number of relatively detailed examples for the interpretations of vibrational spectra. Since the scope of this book is much broader than any of the aforementioned specialized texts, some of the theoretical material needed to be adjusted accordingly. Thus, the quantum mechanics of molecular vibrations, time-dependent perturbations, transition moments, and many other topics are only summarized in this text, and detailed derivations are omitted. For details, the reader is referred to specialized books, such as any one of the many available text books on Quantum Chemistry (see, for example, Levine, *Quantum Chemistry*, Volumes (I) and (II) [3]). For a detailed theoretical background on symmetry aspects, the classical book (Cotton, *Chemical Applications of Group Theory* [4]) is recommended, and the aforementioned books on vibrational spectroscopy for a more thorough treatment of theoretical aspects of molecular vibrations.

Thus, the present book does not supersede any of the classical texts, but is a further extension of them, and intends to bring the reader to a more practical and up-to-date level of understanding in the field of vibrational spectroscopy. Subjects such as Raman spectroscopy, which has become a major area of research in vibrational spectroscopy, is not treated as an afterthought, but experimental and theoretical aspects are discussed in detail. Items of historical significance, such as the Toronto arc for excitation of Raman spectra (which was actually mentioned as a viable source for Raman spectroscopy in a recent text), or the manual solution of the vibrational secular equation, have been banished from this book. Instead, modern experimental aspects, such as multichannel Raman instrumentation, time-resolved and resonance Raman techniques, nonlinear Raman effects, and Fourier transform infrared and Raman techniques are introduced. In addition, computational methods for the calculation of normal modes of vibration are treated in detail in this book.

One chapter is devoted to the biological applications of vibrational spectroscopy. This is a rapidly developing field, and perhaps the most fascinating, for the molecules are often very large and difficult to study due to low solubility and solvent interference. It is in this area that the enormous progress of modern vibrational spectroscopy can best be gauged, since this field is not even discussed in the books of 30 or 40 years ago. The final chapter is devoted to a new branch of vibrational spectroscopy carried out with circularly polarized light. The new techniques introduced here combine principles of vibrational spectroscopy with those of optical activity measurements of chiral molecules. Applications of these techniques to biological molecules, and to simple chiral systems, are presented.

The author would like to thank his colleague, Prof. John Lombardi from the Department of Chemistry, City University of New York, City College, for his encouragement about this book, and for correcting a large number of errors in the original manuscript. The Graduate Spectroscopy class (U761) at the City University of New York in Spring, 1992, also was instrumental in pointing out inconsistencies in the manuscript, when an early version was used for the first time. The author is grateful for the input he received from these students.

New York, January 1993

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