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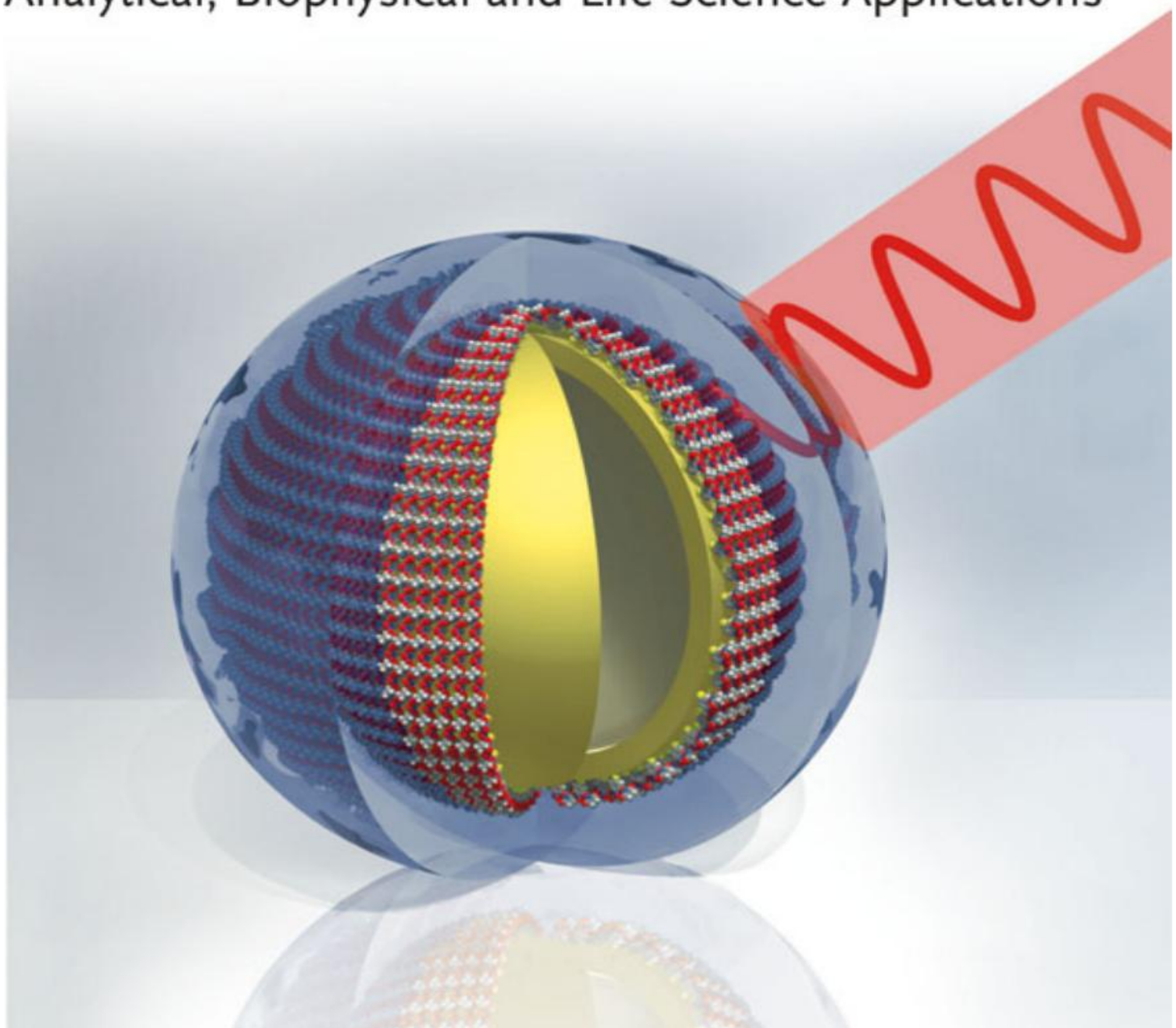
# Surface Enhanced Raman Spectroscopy

## Analytical, Biophysical and Life Science Applications



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# Surface Enhanced Raman Spectroscopy

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# *Foreword*

More than 80 years since the discovery of the Raman effect have passed and Raman spectroscopy has become one of the most important methods within the various methods of analysis and structural determinations. Certainly, the discovery of the laser in 1960 has opened up new horizons for Raman spectroscopy and brought several new useful techniques. One of the most interesting and significant findings in this field is undoubtedly surface-enhanced Raman scattering (SERS) which was discovered in 1977. Within this phenomenon, molecules adsorbed onto metal surfaces under certain conditions exhibit an anomalously large interaction cross section for the Raman effect. It might be thought that a subject originated more than three decades ago would be virtually exhausted by now, but nothing could be farther from truth. The recent developments in SERS have led to large increases in the sensitivity of SERS measurements and have enabled new phenomena to be observed and applied. SERS measurements are expected to become increasingly important in chemistry, biochemistry, and biophysics.

In the 14 chapters of this book, an authoritative, up-to-date account of the principles and fundamentals of SERS is given including many examples for its applications. The book includes the basic theory for SERS; summarizes the various SERS substrates; discusses quantitative SERS methods with emphasis on reproducibility, stability and sensitivity up to single molecule detection; and describes SERS microscopy, electrochemical SERS, surface enhanced resonance Raman scattering (SERRS), and surface-enhanced hyper Raman scattering (SEHRS), as well as surface- and tip-enhanced coherent anti-Stokes Raman scattering (SE-CARS, TE-CARS). Applications of SERS include the detection of organic pollutants and pharmaceuticals; studies of electron transfer of proteins at membrane models; investigations of microfluidics, quantitative DNA analysis, biomedical applications by means of SERS microscopy, SERS as an intracellular probe; and coupling of SERS with various separation methods (e.g. liquid or gas chromatography).

The abundant references provide ready access to the original research literature. As the field of SERS has sufficiently matured during the past decades, the danger of rapid obsolescence for this book is less. The subject matter, however, still offers plenty of opportunity for further exploration and exploitation. In my opinion this book, which clearly expresses the current excitement in this extremely active research area, will make a substantial contribution to the further growth of an increasingly important subfield of vibrational spectroscopy.

Professor Schlücker, editor of this volume, is one of the leading researchers working currently in the SERS field. As chemist at the University of Würzburg, Germany, and the National Institutes of Health, Bethesda, USA, and now as physicist at the University of Osnabrück, Germany, he has played a major role in introducing a few important new experimental techniques of SERS (e.g. the direct and label-free SERS detection of solid-phase bound compounds; immuno-SERS microscopy with nanoparticle probes). He is well qualified to present this book to the scientific community.

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# *Preface*

The field of surface-enhanced Raman scattering (SERS) is currently undergoing a very dynamic development and many novel directions are rapidly emerging. The aim of this book is to provide an overview of current exciting topics in SERS, focusing on analytical, biophysical and life science applications. International leaders in their respective research areas have contributed to this volume. Their original scientific background and training is quite diverse, ranging from bioorganic chemistry to physical chemistry and solid state physics — in my opinion, this directly reflects the highly multidisciplinary nature of SERS applications, a prerequisite for original and pioneering research between the boundaries of traditionally distinct disciplines. The selection of the scientific topics covered in the 14 chapters is naturally subjective and I must certainly apologize to those who have not received the opportunity to contribute to this edition.

This monograph is intended to be useful for both the newcomer with no or little background in Raman/SERS spectroscopy as well as for the experts in the field who are interested in achieving a quick overview as well as in-depth information on specific subjects.

The first part of this book (Chapters 1–3) lays the foundation for the entire book by providing important theoretical and practical background. Topics are the basic electromagnetic theory of SERS, various aspects of metal colloids as plasmonic nanostructures and practical considerations for quantitative SERS. The second part (Chapters 4–14) covers various analytical, biophysical and life science applications of SERS. Chapters 4 through 8 describe analytical applications of SERS, including single-molecule and trace detection, sensors for detecting organic pollutants based on host–guest systems as well as the detection of pharmaceuticals. Two chapters describe the promising combination of SERS with other analytical techniques such as separation methods and microfluidic platforms for lab-on-a chip detection. Chapters 9 and 10 cover spectroelectrochemistry as a classical and important topic in SERS. After an introduction into the theory and experimental setups for combining SERS with electrochemistry, applications to several biological molecules are summarized. Biophysical applications of spectroelectrochemistry with SERS are focused on the electron transfer in membrane models, in particular cytochrome c on coated electrodes. Chapters 11 through 14 report on recent life science applications of SERS. Quantitative DNA analysis with immense multiplexing and ultrasensitive detection capabilities is demonstrated by surface-enhanced resonance Raman scattering (SERRS). Selective protein localization in cells and tissue specimens via SERS microscopy requires the design and fabrication of functionalized metal colloids for labeling target-specific ligands such as antibodies. Information on intracellular biochemical composition and physiological conditions is accessible via one- or two-photon excited SERS in a label-free approach in conjunction with microscopy. Surface- and tip-enhanced coherent anti-Stokes Raman scattering (CARS) as advanced microspectroscopic techniques with sub-diffraction limited spatial resolution together with first applications to DNA are discussed in the last chapter.

I would like to thank all authors for their hard work and commitment to contribute their chapters. This international and multidisciplinary book project would not have been possible without their dedication. The support from Lesley Belfit (Wiley-VCH) and Manfred Köhl (now Thieme) is greatly appreciated. Thanks to Wolfgang Kiefer for his foreword – many of his former students including the editor have contributed to this book. Finally, I would like to thank my wife Uta-Maria, our sons Jan and Henrik, my parents

Marianne and Eberhard as well as my group members for their continuous support.

Osnabrück, August 2010

*Sebastian Schlücker*

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# 1

# *Basic Electromagnetic Theory of SERS*

***Pablo G. Etchegoin and Eric C. Le Ru***

## 1.1 Introduction

This chapter is aimed at introducing the newcomer to the field of surface-enhanced Raman spectroscopy (SERS), and is not intended to supplant the already available exhaustive literature in the field either in the form of review articles [1, 2] or books [3, 4]. As a technique, SERS is relatively exposed to the dangers of specialization due to its (intrinsic) multidisciplinary nature. The technique is becoming widespread and is finding new and exciting horizons in analytical chemistry [5–7], biology and biotechnology [8–12], forensic science [13, 14] and in the study of artistic objects [15–17]. While this is in many ways an advantage, it is also a handicap in the sense that scientists approaching the technique from a more ‘biological’ or ‘applied’ aspect might not have the appropriate background (or predisposition) to venture into the depths of electromagnetic theory and to understand the basic concepts of the theory of plasmon resonances in metallic nanostructures. This could be particularly true for students in the biotechnology field, who might find it desirable to have access to the elementary concepts (with a bare minimum of mathematics) but with enough insight to understand what they are actually doing in the lab. We believe that the success and use of the technique — in an environment which is by nature multidisciplinary — will be more effective if accessible presentations of the basic principles aimed at broader audiences are available at all times (and reviewed over prudent periods of time). This chapter (hopefully) fulfils part of that requirement.

This chapter is organized as follows: in Section 1.2, we introduce the basic principles of plasmon resonances and their associated field enhancements. Section 1.3, on the other hand, looks at the field enhancement distribution and localization produced by these plasmon resonances, while Sections 1.4 and 1.5 study the origin of the enhancement factor (EF) and its characteristic magnitude. Finally, Section 1.6 presents some conclusions and summarizes several main concepts.

# 1.2 Plasmon Resonances and Field Enhancements

## 1.2.1 Optical Properties of Simple Metals

None of the modern optical techniques such as surface-enhanced fluorescence (SEF) [18–20], surface plasmon resonance spectroscopy [21–23] or SERS itself [1, 4] would exist without the particular optical properties of *coinage metals* (with silver (Ag) and gold (Au) standing out as the most useful ones). The first obvious question is then what is it that makes the optical properties of metals so interesting? Hence, it is worth spending a few paragraphs on the topic of the optical properties of *bulk metals* such as Ag and Au to understand why they are so interesting, and why we use them in the aforementioned techniques.