Military University of Technology

Institute of Optoelectronics

Col. Bartłomiej Jankiewicz, PhD, DSc
Institute of Optoelectronics
Military University of Technology
gen. S. Kaliskiego 2
00-908 Warsaw

Warsaw, April 29th, 2025

Review

of the Ph.D. Thesis of Abdolvahab Amirsalari, M.Sc., entitled

„Wavelength-scanned surface-enhanced Raman excitation spectroscopy and imaging – the development of the method and its application"

submitted to the Scientific Council of the Institute of Physical Chemistry, Polish Academy of Sciences in Warsaw.

Legal basis for preparing the review:

The review was carried out based on the resolution of Scientific Council of the Institute of Physical Chemistry, Polish Academy of Sciences in Warsaw, Poland of February 25, 2025, following the Act of July 20, 2018 - the Law on Higher Education and Science (as amended), at the request of the Deputy Director of Scientific Affairs of the Institute of Physical Chemistry, Polish Academy of Sciences, prof. Jacek Gregorowicz, of February 28, 2025, and the Ph.D. thesis entitled "Wavelength-scanned surface-enhanced Raman excitation spectroscopy and imaging – the development of the method and its application".

1. General characteristics of the Ph.D. thesis

The Ph.D. thesis of Abdolvahab Amirsalari was prepared under the supervision of Prof. Jacek Waluk, leader of the "Photophysics and spectroscopy of photoactive systems" Research Group of the IPC PAS, and co-supervision of Dr. Sylwester Gawinkowski, leader of the "Molecular Nanophotonics" Research Group of the IPC PAS. This thesis describes the results of extensive studies on developing and applying wavelength-scanned Surface-Enhanced Raman Scattering (SERS) and Surface-Enhanced Resonant Raman Scattering (SERRS) techniques. The focus of studies was on the excitation profiles of various molecules, with a detailed analysis of their interactions with gold nanorods used as SERS substrates. An emphasis is placed on understanding enhancement mechanisms at the ensemble and single-nanostructure levels. The research includes:

- Design and construction of a SERS microscopy set-up.
- Detailed study of excitation profiles across multiple probe molecules.
- Simulation-based interpretation of electromagnetic (EM) enhancement.
- SERRS at the single-nanoantenna and hot-spot level.
- Consideration of single-molecule detection regimes.

The research and analyses presented and described in the thesis fall within the thematic area of the scientific discipline of chemical sciences.

2. Evaluation of the Ph.D. thesis

Assessment of the structure of the Ph.D. thesis and information about its components

The Ph.D. thesis of Abdolvahab Amirsalari, submitted for evaluation, is extensive and covers 239 pages. The thesis is generally characterized by a clear and logical layout typical of Ph.D. theses and proper content division into chapters, each building progressively on the previous ones. The language of the thesis is clear, scientific, and accessible to readers familiar with spectroscopy or nanophotonics.

The main content of the thesis is preceded by abstracts in Polish and English, followed by acknowledgments, a list of the Author's scientific publications and presentations, a preface to the thesis, and the table of contents. The introductory part of the thesis, 30 pages long, contains two chapters. Chapter 1 briefly introduces SERS and provides an overview of the thesis. Chapter 2 presents the theoretical foundation of normal, resonance, and surface-enhanced Raman scattering. In Chapter 3, the Author describes the key objectives of the thesis. The following four chapters, 155 pages long in total, describe the research conducted by the Author, including the research methodology (experimental setup and data analysis) and results of carried out studies on SERS excitation profiles for various molecules, SERRS excitation profiles on single nanorods and single hot-spots, and detectability at the single-molecule regime. Each chapter discussing the results of the Author's work begins with a brief description explaining the aim of the investigation and ends with the conclusions. Chapter 5 describes the first set of results from SERS and SERRS excitation studies across different molecules, including average SERS and SERRS excitation profiles on a large substrate area. Chapter 6 focuses on SERRS excitation studies on single nanostructures, including single nanorods and hot-spots. Finally, Chapter 7 describes how the results of the previous chapters impact detectability at low concentrations and in the single-molecule regime. In the last two chapters, the author provides suggestions for future research in the field and a conclusion of the research results described in the thesis. The last section of the thesis is Bibliography, which on 31 pages contains a list of literature to which the Author refers in the thesis.

The text contains numerous figures (123) and tables (4), which clearly present the achievements and accomplishments of the Author of the thesis. Figures play a strong role in presenting complex data generated during research studies carried out by the Author.

Evaluation of the bibliography in the Ph.D. thesis

The bibliography consists of 365 references related to the subject of the work and includes articles, conference proceedings, books, Ph.D. and M.Sc. theses, and websites. In general, the Author correctly selected the literature, with some exceptions mentioned below. Still, I have reservations about the few publications from the last few years, as ca. 21% of cited publications were published since 2015. The literature review could possibly be expanded with more recent developments post-2020, especially in biosensing contexts.

I have found some issues related to the bibliography, including missing information in some references or incorrect information (e.g., references 9, 16, 23, 29, 96, 100). However, my strong concern is about references I could not find in any databases or journals' websites, including at least the following references: 6, 7, 8, 10, 11, 12, 13, 20, and 24. The author must certainly explain this issue.

Indication and assessment of the Ph.D. candidate's thesis objective

This Ph.D. thesis aims to deepen the understanding of Surface-Enhanced Raman Scattering (SERS) and Surface-Enhanced Resonant Raman Scattering (SERRS) excitation profiles for improved analytical and photophysical applications. The key objectives of a Ph.D. candidate's thesis are:

- Investigate SERS and SERRS excitation profiles on aggregated nanorods to better understand enhancement mechanisms through combined experimental and simulation approaches.
- Analyse single nanoparticle behaviour by studying SERRS excitation profiles at the single-nanoparticle and hot-spot levels, revealing molecular interactions and electronic state variations.
- Enhance single-molecule detectability by improving SERS and SERRS sensitivity in the low-concentration regime.

The thesis seeks to expand fundamental knowledge and applications of SERS and SERRS techniques by achieving these goals. In my opinion, the author formulated the key objectives correctly, justified them, and ultimately achieved them.

Indication and evaluation of the research methods used

While carrying out the research studies, the results of which became the basis for the preparation of the submitted Ph.D. thesis, the candidate used many research methods, which were selected correctly. The research methods were described in detail, and their use was justified in Chapter 4. The author provided a detailed explanation of material sources, chemicals, gold nanorods, and dyes, followed by the fabrication and characterization of different SERS substrates and protocols for registering various probes to the SERS template.

Gold nanorods used in the studies were either synthesized or purchased (three types of nanorods). The nanorods and SERS substrates fabricated using them were characterized using scanning electron microscopy (SEM). Additionally, the surface topography of the substrates was characterized using atomic force microscopy (AFM) to evaluate the distribution and compactness of nanorods dispersed on the coverslip. The optical properties (extinction spectra) of substrates coated with a uniform layer of nanorods were measured using absorption spectroscopy. The dyes used in the studies, with some exceptions, were characterized using absorption and fluorescence spectroscopy. Selected dyes (Cy5Pc and CY6Pc) were additionally investigated using magnetic circular dichroism (MCD) spectroscopy.

The Author used a custom-built SERS microscopy setup to investigate SERS excitation profiles and radiation patterns, record SERS signals from single nanoantennas, and perform dark-field and single nanoantenna scattering measurements. The measurements of typical SERS spectra were carried out using the commercial Raman system, the InVia Renishaw Raman spectrometer. Chapter 4 details the microscopic system and its implementation for SERS excitation measurements, as well as numerical tools and methods for analysing experimental data and simulations. The Author used finite-difference time-domain (FDTD) simulations to support the experimental results. In the evaluated Ph.D. thesis, the FDTD simulations were used to estimate EM enhancement factors for densely packed nanorods on coverslips, employed as SERS substrates, and to evaluate enhancement factors for individual nanorods. The FDTD simulations were also used to investigate the scattering and extinction phenomena associated with these nanorods.

Assessment of the part of the Ph.D. thesis concerning the discussion of research results

The results of the experimental studies conducted using the methods described in Chapter 4 are extensively and clearly characterized in Chapters 5, 6, and 7, and then summarized in the final conclusions in Chapter 9. Additionally, Chapter 8 provides suggestions for future research in the field.

Chapter 5 presents the first set of results from SERS and SERRS excitation studies across different molecules, including average SERS and SERRS excitation profiles on a large substrate area. The chapter begins with detailed photophysical studies of selected molecules, including Pc and its derivatives (TTPc, CY5Pc, and CY6Pc), 2-naphtalenethiol (a non-resonant probe to assess EM enhancement trends), and malachite green (resonant probe commonly used in SERS studies and chosen for its distinctive photophysical properties), to enhance understanding of probe behaviour in different states and environments. The chapter also contains an analysis of the extinction spectra of the used substrates. The observed extinction behaviour was attributed to plasmon resonance effects. It was also concluded that the longitudinal plasmon mode dominates scattering, absorption, extinction, and EM enhancement. The following sections of Chapter 5 describe SERS excitation studies, which are supported by numerical simulation results. Numerical simulations were performed to model substrates similar to SERS substrates, interpret the SERS excitation profiles, and assess their electromagnetic behaviour. The Author compares the SERRS excitation profiles of the molecules under study with their corresponding absorption spectra to better understand their SERRS behaviour. The investigative approach was extended by thoroughly examining the SERRS excitation profiles for different vibrational bands and the integrated SERRS excitation profile across multiple vibrational bands.

Chapter 6 focused on SERRS excitation studies on single nanostructures, including single nanorods and hot-spots. The studies aimed to determine whether a limited population of nano environments can provide improved spectral resolution of SERS excitation profiles, which is essential for both detectability and spectroscopy. Similarly to Chapter 5, data analysis was supported by numerical simulation results. In this chapter, SERRS excitation profiles at both single-nanorod and hot-spot levels were presented, and their spectral behaviours and underlying mechanisms were compared to profiles from an area of aggregated nanorods. The described findings highlight the influence of nanoenvironment variations on SERRS excitation profiles, with significant distinctions between individual nanorods, aggregated nanorods, and hot-spots. As indicated by the Author, results described in Chapters 5 and 6 pave the way for higher sensitivity in SERS as a function of frequency.

Chapter 7 focuses on studies of one practical application: detecting low concentrations of analytes and single molecules. Firstly, the review of single-molecule events is provided, which, according to the Author, indicates that, “except for a limited number of molecules known for their high Raman cross-sections and capable of detection without resonant conditions, the majority of other molecules have been measured, consciously or unconsciously, under resonant conditions". In the experimental studies, the Author used two probes, CY5Pc and CY6Pc, on templates of aggregated nanorods. The Author successfully detected single-molecule events when the excitation was adjusted to the frequency that provides maximum signal due to the resonance Raman effect.

The chapters of the Ph.D. thesis concerning the discussion of results confirm the good organization of the planned and conducted research and the ability to systematize activities leading to achieving the formulated objectives. In my opinion, the strengths of the thesis's part dedicated to discussing research results lie in a deep theoretical background paired with rigorous experimental validation and effective use of numerical modelling to support interpretations of the results. Worth noticing is the breadth and depth of molecular systems studied under varied resonant conditions. A thoughtful discussion of experimental challenges (e.g., fluorescence, stability, laser power) in real-world detection scenarios should also be noted. In conclusion, I would like to emphasize the wide scope of Abdolvahab Amirsalari's research, which used many research methods and comprehensively analysed the results.

Information on the practical application of the obtained research results

The Raman spectroscopy, similar to other chemical analysis techniques, can identify, quantify, and characterize various substances. However, it has several advantages that make it special and widely used in many areas to perform quality control, sample identification, materials characterization, and general investigation of physical and chemical properties. Among the advantages of Raman spectroscopy is that it is a non-contact technique, little to no sample preparation is required, measurements can be made through transparent packaging, it can examine microscopic structures, can easily measure aqueous solutions, powerful handheld devices are available, data quality is excellent, and spatially resolved analysis by microscopy and imaging can be carried out.

Surface-enhanced Raman spectroscopy has an advantage over normal Raman spectroscopy in its ability to significantly enhance the Raman signals of molecules, which is important in the analysis of low concentrations of the analyte. The disadvantages of SERS are mainly related to the need for the use of nanostructured SERS substrates and the issues related to their use, such as fragility of SERS substrates, limited reusability, and inhomogeneity and irreproducibility of signals within a substrate. SERS has been the subject of extensive theoretical and experimental research, which, together with the continuous development in nanotechnology, has significantly broadened the scope of SERS and made it a hot research field in many areas of science. Despite numerous studies, SERS has not been developed into a routine analytical technique, and efforts continue to address the problems that prevent its real-world application.

In my opinion, any research studies related to SERS and its potential practical applications are bringing this technique closer to real-life use by various professionals relying on chemical analysis. This also applies to the results described in the evaluated Ph.D. thesis. In the introduction, the author described some problems with the potential practical use of SERS and SERRS techniques and indicated those issues that were targeted in his research studies. In conclusion to Chapter 5, the author stated that "understanding the SERS and SERRS excitation profiles based on the spectral range and the factors influencing the reduction in signal strength can aid in optimizing SERS measurements and advancing the field of SERRS towards more precise and reliable analyses in diverse applications". Of great importance for practical use is also the observation that matching the laser frequency to the electronic transition of the molecule that may undergo a shift on the metal surface, compared to in solution, can significantly enhance the SERS signal by several orders of magnitude. It was also shown that for the investigated molecules, when the excitation was adjusted to the frequency that provides maximum signal due to the resonance Raman effect, single-molecule events were detected.

In conclusion, the evaluated Ph.D. thesis reports on the research results, which advance understanding of SERS and SERRS excitation profiles and low concentration and single-molecule detectability. As indicated by the Author, the findings of this work lay a foundation for developing highly sensitive analytical tools applicable to a wide range of practical applications, from medical diagnostics to environmental sensing.

Assessment of whether the Ph.D. thesis contains an original solution to a scientific problem

Mr. Abdolvahab Amirsalari certainly achieved the objectives of his Ph.D. thesis. This thesis represents an important contribution to nanophotonics and molecular spectroscopy. Its mix of innovation in instrumentation, thorough experimental data, and deep theoretical analysis positions it as a valuable reference in academia and applied research. Taking into account their scientific nature, I state that the research work conducted by the Author constitutes an original solution to a scientific problem. The evaluated Ph.D. thesis advances methodological and application-focused frontiers in SERS/SERRS, particularly in single-molecule detection, which has high potential for application in biosensing and analytical chemistry. The thesis stands out for its comprehensive spectral mapping of excitation profiles using a broad range of excitation wavelengths, far more extensively than previously documented. The study of SERRS on single nanoantennas and hot-spots is relatively rare and adds significant value to the field.

Assessment of whether the Ph.D. thesis presents the candidate's general theoretical knowledge in the discipline or disciplines and the ability to conduct scientific or artistic work independently

In my opinion, the work described in the Ph.D. thesis falls within the thematic area of the scientific discipline of chemical sciences. It is also written correctly from the point of view of methodology. The introductory part, containing the theoretical aspects of the work, description of research methods, conducted studies, and discussion of results presented by Mr. Abdolvahab Amirsalari, indicate that the Ph.D. candidate has general and well-grounded theoretical knowledge in the chemical sciences. The evaluated Ph.D. thesis demonstrates the candidate's ability to conduct research independently.

Information about any irregularities that occurred in the assessed thesis:

The work is generally written in correct language. The concepts and terminology used do not raise any major objections. Below, I have included a few comments regarding the editorial side of the thesis, which do not affect the achievement of the scientific goals of the work. My comments regarding some minor issues are given below:

- The captions under the Figures do not fully reflect their content i.e, Figure 4.2.
- The author stated, "Analyzing the data from AFM indicated that the ratio of the nanoparticle surface area to the substrate surface area was nearly one-to-one… Figure 4.4" – however, it does not seem correct based on Figure 4.4.
- Missing information in the text regarding LSP and TSP peaks of synthesized gold nanorods.

3. **Final assessment of the Ph.D. thesis**

In my opinion, Mr. Abdolvahab Amirsalari has achieved the research objectives that are the subject of his Ph.D. thesis. The summary of research results included in the thesis is documented. The formulated thesis has been proven based on the conducted literature study and the performed and correctly interpreted results of the own research. The manner of presenting and developing the research results indicates that the Author of the thesis has mastered the research workshop necessary to carry out the work to a very good degree and has demonstrated the necessary knowledge in the field of chemical sciences, research planning, and methods of developing results. The Author correctly formulated conclusions of cognitive significance and skillfully generalized them. Taking into account the cognitive and application relevance of the work, the manner of implementing the research program, the form of development and presentation of the results of the performed research, as well as the presented conclusions, I state that the Ph.D. thesis of Mr. Abdolvahab Amirsalari, entitled "Wavelength-scanned surface-enhanced Raman excitation spectroscopy and imaging - the development of the method and its application" meets all the requirements for Ph.D. thesis specified in art. 187 of the Act of 20 July 2018 - The Law on Higher Education and Science (as amended), and I request that he be admitted to the further stages of the doctoral process.

....
(Reviewer's signature)