

Label-Free Detection of Exosome Components via a Novel SPR-Raman Platform

Yong Ai Wu¹, Nan-Fu Chiu^{1,2}, Jou-Chen Lin¹, Tien-Hsiung Ku^{3,4,5}

¹ Institute and Undergraduate of Electro-Optical Engineering, National Taiwan Normal University, No. 88, Sec. 4, Ting-Chou Road, Taipei 11677, Taiwan

² Research Center for Applied Sciences, Academia Sinica, 128 Academia Road, Section 2, Nankang, Taipei, 11529, Taiwan

³ Department of Applied Chemistry, Providence University, Taichung 43301, Taiwan

⁴ Department of Anesthesiology, Changhua Christian Hospital, Changhua 500209, Taiwan

⁵ Artificial Intelligence Development Center, Changhua Christian Hospital, Changhua 500209, Taiwan
nfchiu@ntnu.edu.tw

Summary:

This study introduces a novel biosensing platform that integrates surface plasmon resonance (SPR) and Raman spectroscopy for the quantification of exosomes and the biochemical profiling of their contents, with promising implications for early cancer diagnostics. We successfully determined the concentrations of three exosomal transmembrane proteins—CD63, CD9, and CD81—while simultaneously characterizing the internal molecular cargo using Raman spectroscopy. This dual-modality approach enables real-time, quantitative detection and comprehensive structural analysis of exosomes.

Keywords: surface plasmon resonance (SPR), Raman spectroscopy, exosomes, cancer.

Background, Motivation and Objective

The global epidemiological landscape of lung cancer is undergoing a significant paradigm shift. Although tobacco use remains the dominant risk factor, a growing incidence among never-smokers has been increasingly reported worldwide. Accumulating evidence identifies ambient air pollution, particularly fine particulate matter (PM_{2.5}) and associated carcinogens, as a critical contributor to this trend. Recognizing its impact, the International Agency for Research on Cancer has classified outdoor air pollution as a Group 1 human carcinogen [1,2]. These findings highlight the urgent need for effective early diagnostic strategies. Exosomes have emerged as promising non-invasive biomarkers for precision diagnostics [3]. However, their nanoscale size and structural complexity present analytical challenges. To address these limitations, this study proposes an integrated biosensing platform combining surface plasmon resonance (SPR) with Raman spectroscopy [4,5]. This SPR-Raman system enables real-time quantification of exosomes while providing detailed biochemical profiling of their molecular cargo. By integrating quantitative and qualitative analyses, this multi-modal approach enhances sensitivity, specificity, and diagnostic accuracy for early lung cancer

detection, particularly in high-risk populations exposed to environmental pollutants.

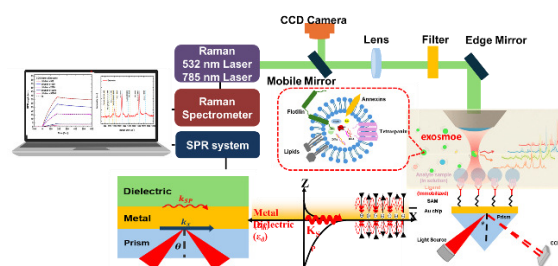


Fig. 1. Schematic representation of the integrated SPR and Raman spectroscopy platform for real-time exosome quantification and molecular profiling.

Description of the New Method or System

In this study, a novel biosensing platform integrating a microscope-based surface plasmon resonance (SPR)-Raman system was developed for real-time, highly sensitive, and label-free detection of exosome biomarkers. The system architecture is illustrated in Figure 1. This enables precise, real-time monitoring of binding kinetics and analyte concentration, while also providing quantitative information on mass accumulation at the sensor surface. However, SPR alone cannot resolve detailed molecular structures or functional groups. To address this limitation, Raman spectroscopy is incorporated to

obtain molecular fingerprint information. Using 532 nm excitation lasers, the microscope-based Raman module detects characteristic vibrational modes, enabling label-free identification of biochemical components without additional labeling. During operation, SPR is first used to monitor biomolecular immobilization and interaction dynamics, followed by Raman analysis to characterize biochemical composition. This integrated platform combines quantitative and qualitative capabilities, offering a comprehensive approach for cancer biomarker detection, early diagnosis, and therapeutic monitoring.

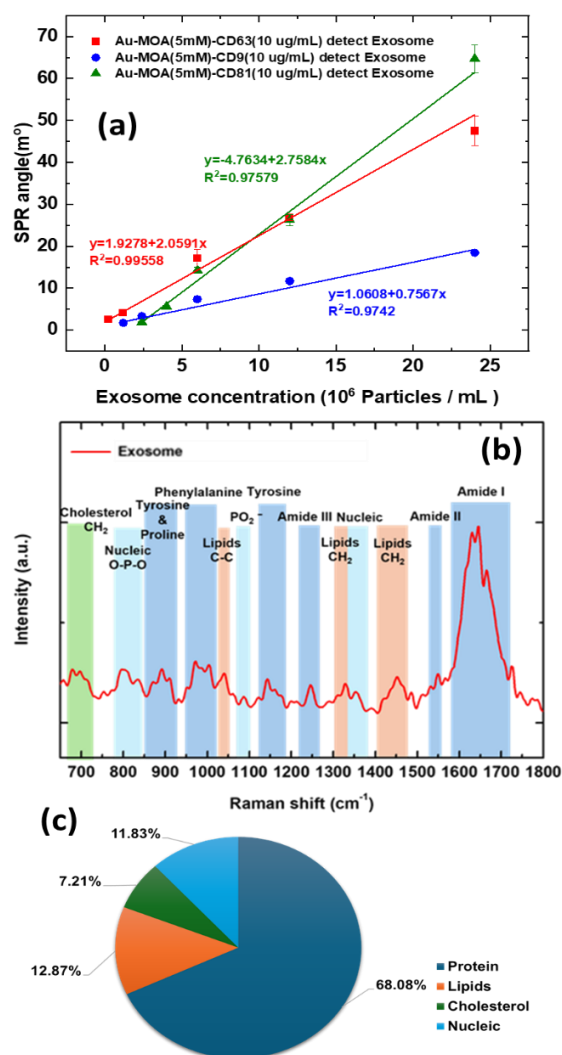


Fig. 2. (a) SPR sensor response demonstrating a linear correlation between biomolecular interactions of exosome surface markers (CD63, CD9, and CD81) and variations in exosome concentration. (b, c) Raman spectra of exosomes acquired using a 532 nm excitation wavelength, together with corresponding

spectroscopic characterization and quantitative analysis of molecular composition.

Results

Through SPR analysis, we preliminarily confirmed the presence of key exosomal transmembrane proteins, including CD63, CD9, and CD81, at a measured concentration of 2.4×10^6 particles/mL. As shown in Figure 2(a), the SPR sensor demonstrates excellent linearity and a low limit of detection (LOD), indicating high sensitivity and reliable quantitative performance. Figures 2(b) and 2(c) present real-time Raman spectral and compositional analyses of the exosome samples. These results reveal distinct variations in the molecular signatures associated with transmembrane proteins (CD63, CD9, and CD81), reflecting differences in exosome surface composition. The integrated SPR-Raman platform enables simultaneous real-time quantification and biochemical characterization, allowing precise evaluation of protein expression levels and surface binding affinities. Overall, the results provide detailed insights into the molecular characteristics of exosomes. This capability facilitates more accurate discrimination between healthy individuals and those in different physiological or pathological states, highlighting the platform's potential for disease diagnostics and biomarker profiling.

References

- [1] International Agency for Research on Cancer (2013). Outdoor air pollution a leading environmental cause of cancer deaths. Lyon: IARC Press.
- [2] C.B. Berg et al., Air pollution and lung cancer: mechanistic insights from epidemiology and biology, *Nature* 607, 241-249 (2022); doi:10.1016/j.jtho.2023.05.024
- [3] H. Valadi et al., Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, *Nature Cell Biology*, 9, 654-659 (2007); doi:10.1038/ncb1596
- [4] C.-L. Chiang, N.-F. Chiu, J.-H. Yang, D.-J. Chang, C.-W. Lin, Layer-by-Layer Characterizations of Protein Complexes by Surface Plasmon Resonance Enhanced Raman System-Detection of Heparin-Platelet Factor IV Complexes as an Example, *Sens. Actuators B Chem.* 452, 139461 (2026). doi:10.1016/j.snb.2026.139461
- [5] J.-C. Lin, N.-F. Chiu, Development of an SPR-Raman biosensor for early lung cancer biomarker detection, *Proc. SPIE, Optical Sensors 2025*, 135270S (23 May 2025). doi:10.1117/12.3056355