

# Multiplexed SERS Analysis of Extracellular Vesicles for Colorectal Cancer Diagnosis

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## Abstract

Extracellular vesicles (EVs) have emerged as promising tumor biomarkers; however, their clinical application remains limited due to the insufficient sensitivity and multiplexing capability of existing EV analysis methods, particularly when only small volumes of body fluids are available. Here, we present a multiplexed EV analysis platform based on a surface-enhanced Raman scattering (SERS) immunoassay. The platform integrates a crossed gold nanowire substrate with gold nanoparticle-based SERS tags, generating strong electromagnetic enhancement for highly sensitive and multiplexed EV detection.

The proposed assay achieved an ultralow limit of detection of 10 EVs in a 5  $\mu$ L sample volume and enabled simultaneous detection of four colorectal cancer (CRC)-associated EV surface proteins. By combining the signals from these four EV markers, healthy controls were clearly distinguished from CRC patients with an accuracy of 92%. A weighted composite score significantly improved diagnostic performance compared with individual EV markers. In addition, the feasibility of applying this SERS-based EV analysis for monitoring post-surgical recurrence in CRC patients was demonstrated.

Overall, this multiplexed SERS-based EV analysis platform provides a highly sensitive and robust approach for EV profiling from small-volume samples, offering new opportunities for accurate cancer diagnosis and post-operative disease monitoring.