Project scientific goal. The incidence of cancer is a global problem; according to GLOBOCAN, the

incidence will reach 28.4 million patients worldwide by 2040. Among death-causing tumors/cancers, **salivary gland tumors** (SGTs) are challenging to diagnose because of their stochastic nature, heterogenic histopathological, and genetic diversity (largely unexplored variety of entities and subtypes). Therefore, establishing a novel diagnostics approach through a combination of effective cancer detection/diagnosis methods and treatment modalities for clinical uses is a formidable need and challenge. Here, we propose an alternative realization of diagnostics based on the surface-enhanced Raman spectroscopy (SERS) technique for salivary gland tumors-derived Exosomes. Exosomes reflect the phenotypic state of the cells and their metastatic potential, making them key tumor detection and treatment biomarkers. The Project's scientific goal is to solve basic and applied problems significant for developing novel dielectrophoretic isolation and aptamers-based recognition units using **SERS** techniques to investigate the exosomes and exosomal proteins associated with SGTs comprehensively. SERS spectroscopy, which has a unique advantage for rapidly detecting clinically relevant changes supported by the high sensitivity, selectivity, elimination of expensive reagents, and time-consuming sample preparations, will overcome the significant drawbacks in EV analysis.

Project research methodology. The Project is *interdisciplinary*, and its *innovative character* involves the development of a novel DEP/SERS-mediated aptasensors to isolate/capture EVs from blood and saliva samples and their molecular characterization for the first time in one technology. During the subsequent steps of the Project, we will design and characterize the SERS-active nanostructures and optimize them regarding sensitivity, reproducibility, and stability of EV SERS responses. Their morphology will be visualized using AFM, SEM techniques, and chemical analysis using XPS measurements. In collaboration with consortium teams, we will design and synthesize the aptamers with high affinity to selected exosomal proteins. The BLI technique, structural modelling, and molecular docking will be adopted for aptamer-exosome interaction studies. Then, we will develop a novel method of SGT's exosome isolation and analysis called SERS-DEP-(Lab-on-Chip). The dielectrophoresis will be adopted to separate exosomes from human fluids and deposit them on the SERS platform for SERS probing. To assess the conditions under which the optimum effect of exosome deposition will be determined, the numerical calculations will be completed. We will perform collaborative fluorescence measurements with our partners to unravel the specific biochemical properties of vesicles and verify the efficiency of exosome isolation. The SERS/DEP devices will be programmed to both be label-free for whole exosomes and labeled for tumor-related biomarkers of SGT's exosome analyses, respectively. In the direct SERS-based approach, the biochemical fingerprint of whole exosomes will be determined after their isolation by the DEP effect from human fluids (SERS signals will be recorded directly from exosomes deposited on SERS platform). In the indirect configuration, the SERS signals of the Raman reporters, will contain qualitative and quantitative information on exosome-specific biomarkers (proteins), providing high sensitivity and specificity for detection, and enabling multiplex detection, especially important in samples with low concentrations of EVs. The SERS-based proteomic profiling will be validated by complementary mass spectrometry. Moreover, the BLI and SERS as dual control systems will be used to characterize developed recognition unit performance and quantify its sensitivity and detectability. We will also use traditional chemometric methods and classical machine-learning models to extract the biochemical information encoded in the complex SERS signature**.** **Impact of the Project results.** The developed tools enable a new method for the isolation, identification, enumeration, and molecular characterization of EVs, providing the opportunity for a real-time "liquid biopsy" that opens new ways for cancer diagnostics. In the future, the project results will facilitate the monitoring of patients' clinical outcomes.