Single Extracellular VEsicle Nanoscopy-Universal Protocol (SEVEN-UP): accessible imaging platform for quantitative characterization of nanoscopic particles

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All cells shed nanoscale particles called extracellular vesicles (EVs). EVs have key roles in intercellular communication, and they have recently emerged as exciting therapeutic modalities, delivery vectors, and biomarker sources. Despite these important features, EVs have been challenging to reproducibly characterize because they are heterogeneous in size, origin, and molecular content. To advance EVs in biology and biomedicine, tools for biophysical assessment of individual EVs and other nanoparticles that do not require highly specialized equipment are urgently needed. We quantified individual EVs by combining affinity isolation with super-resolution radial fluctuations (SRRF) microscopy and new analysis algorithms supported by machine learning[1]. This combined approach we called Single Extracellular VEsicle Nanoscopy-Universal Protocol (SEVEN-UP) is compatible with a wide range of microscopes and does not require specialized fluorophores. The workflow was optimized and validated using recombinant and plasma EVs enriched in classical EV markers (e.g., tetraspanins CD9, CD63, and CD81) by correlating SRRF and single-molecule localization microscopy images. For captured EVs, we robustly assessed surface density, size, and molecular content of tetraspanins. Further, using plasma of healthy and cancer patients, we assessed plasma EVs enriched in different glycosylation motifs. Our data point to a unique population of glycosylated EVs in cancer. Altogether, we developed a new and economical tool to characterize the biophysical properties of individual nanoscopic particles. By democratizing single EV imaging, SEVEN-UP may help unravel basic EV biology and move EVs toward clinic.

References:

[1] Saftics, A.; et al. *Analytical Chemistry,* 2025 *in press*