Nanoscale Compartmentalisation *via* Plasmonic Nanohole Arrays for Super-Resolution Digital Assay of Small Extracellular Vesicles

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Nanoplasmonic structures, particularly periodic nanohole arrays (NHAs), offer unique optical properties for sensing and imaging through subwavelength light confinement.[1] While plasmonic NHAs are established as powerful optical biosensors,[2] their potential for enhancing imaging quality remains underexplored. This work introduces a novel application of plasmonic NHAs as an imaging substrate for a single-particle-resolution digital assay for the detection of single small extracellular vesicles (sEVs), which are highly promising biomarkers for liquid biopsy-based cancer diagnosis, especially for early cancer detection.[3] This on-chip solution utilises the localised surface plasmon resonance of the plasmonic NHAs to generate precise illumination patterns for Structured Illumination Microscopy (SIM). Furthermore, the NHAs provide nanoscale compartmentalisation, physically isolating single sEVs and creating a new class of highly sensitive digital assays.

Plasmonic NHAs were fabricated using standard semiconductor techniques.[4] A custom-built 4f microscope with galvanometric beam steering was used to acquire SIM images utilising the on-chip illumination from the plasmonic NHA. Selective excitation of near-field modes across the NHA was achieved through azimuthal and polar adjustments at the objective back focal plane. Super-resolved images were computationally reconstructed from a subset of nine structured illumination frames using a modified blind-SIM algorithm.[5] Fluorescent EVs from a commercial source were confined into the nanoholes using a capillary force-assisted scraping method.[6]

The results show that compartmentalisation of EVs into the holes achieved >90% occupancy, and plasmonic-assisted SIM enabled high-density, super-resolution imaging of these confined EVs. SEM imaging confirmed EV registration within the nanoholes. One-dimensional Fourier transform analysis of fluorescence images showed spectral peaks matching the hexagonal lattice periodicity, further confirming nanohole compartmentalisation. Resolution analysis showed a ~80 nm full width at half maximum (FWHM) and the ability to resolve dimer particles spaced by ~100 nm, which is beyond the capability of diffraction-limited microscopy. This system presents a promising approach for high-resolution digital assays, particularly for early cancer detection using single EV analysis.

References:

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