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Quantitative Detection of Biological Nanoparticles Using Twilight Off-axis Holographic Microscopy: Insights on Complex Formation Between Pegylated Gold Nanoparticles and Lipid Vesicles

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The detection of biological nanoparticles, such as viruses and extracellular vesicles, plays a critical role in medical diagnostics. However, these particles are optically faint, making microscopic detection in complex solutions challenging. Recent advancements have demonstrated that distinguishing between metallic and dielectric signals in off-axis twilight holographic microscopy allows for quantitative assessment and differentiation between metal-biological NP complexes and free metal or biological NPs. In this study, we employ this method to investigate optimal conditions for complex formation through specific interactions between gold nanoparticles (AuNPs) and lipid vesicles, serving as virus mimics. PEGylated AuNPs with 0.06% biotin (5 biotin per AuNP) were modified with streptavidin (StrAv-AuNPs). It was found that a high StrAv excess during modification was required to fabricate functional StrAv-AuNPs. Using waveguide scattering microscopy, surface plasmon resonance, and off-axis holographic microscopy, we demonstrate that this likely stems from biotin inaccessibility within the PEG layer. Furthermore, we show that reducing the molecular weight of non-functional PEG mitigates this issue. Specifically, using 3 kDa PEG and 5 kDa PEG-biotin generates functional StrAv-AuNPs which, regardless of the StrAv:biotin ratio during formation, enable sub-pM limit of detection.