Single-protein holography

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Light scattering by nanoscale objects is a fundamental physical property defined by their scattering cross-section. Over the past decade, a variety of approaches have demonstrated single molecule sensitivity, by interfering the coherent scattering from the object of interest with a reference field. This approach enables mass measurement of single biomolecules in solution owing to the linear scaling of the image contrast with the molecular polarisability. Nevertheless, all approaches to date cannot separate and independently tune the reference and scattered light field, meaning that the underlying polarizability cannot be quantified.

Here, we present a dark-field scattering microscope which, similar to a Mach-Zehnder interferometer, separates the scattering and reference light into separate arms, enabling us to introduce distinct phase shifts in four parallel detection channels, in a fashion that is insentive to absolute pathlength changes, enabling highly sensitive phase measurements. Combination of these four interference images allows us to reconstruct the complex scattering field. We calibrate our instrument with gold nanoparticles and demonstrate detection and mass measurement of single proteins below 100 kDa. Importantly, holographic detection separates the amplitude and phase information, which yields direct information on sample identity and the first experimental determination of the scattering cross-section of single biomolecules.