Evanescent single-molecule biosensing with quantum-limited precision

N. P. Mauranyapin, L. S. Madsen, M. A. Taylor, M. Waleed and W. P. Bowen

Sensors that are able to detect and track single unlabelled biomolecules are an important tool to understand biomolecular dynamics and interactions as well as for medical diagnostics operating at their ultimate detection limits. Recently, exceptional sensitivity has been achieved using the strongly enhanced evanescent fields provided by optical microcavities and plasmonic resonators. However, at high field intensities, photon damage to the biological specimen becomes increasingly problematic. Here, we introduce an evanescent biosensor that operates at the fundamental precision limit due to the quantization of light. This allows a four orders of magnitude reduction in optical intensity, while maintaining state-of-the-art sensitivity. It enables quantum noise-limited tracking of single biomolecules as small as 3.5 nm, and monitoring of surface-molecule interactions over extended periods. By achieving quantum noise-limited precision, our approach provides a path towards quantum-enhanced single-molecule biosensors.

Evanescent optical biosensors that operate label-free and can resolve single molecules have applications ranging from clinical diagnostics to environmental monitoring and the detection and manipulation of viruses, proteins and antibodies. Furthermore, they offer the prospect of providing new insights into motor molecule dynamics and biophysically important conformational changes as they occur in the natural state, unmodified by the presence of fluorescent markers or nanoparticle labels. Recently, the reach of evanescent techniques has been extended down to single biomolecules with dimensions in the single nanometre range by concentrating the optical field using resonant structures such as optical microcavities and plasmonic resonators. These advances illustrate a near-universal feature of precision optical biosensors—that increased light intensities are required to detect smaller molecules or improve spatiotemporal resolution. This increases the photodamage experienced by the specimen, which can have broad consequences on viability, function, structure and growth. It is therefore desirable to develop alternative biosensing approaches that improve sensitivity without exposing the specimen to higher optical intensities.

Here, we demonstrate an optical nanofibre-based approach to evanescent detection and tracking of unlabelled biomolecules that utilizes a combination of heterodyne interferometry and dark-field illumination. This greatly suppresses the technical noise due to background scatter, vibrations and laser fluctuations that has limited previous experiments, allowing operation at the quantum noise limit to sensitivity introduced by the quantization of light, for the first time. The increased information that is extracted per scattered photon enables small nanoparticles and biomolecules to be detected and tracked with high resolution using optical intensities four orders of magnitude lower than has been possible previously. Furthermore, using this sensing platform we show that surface changes can enhance the performance of single-molecule biosensors by increasing the molecular scattering cross-section.

The nanofibre sensor is immersed in a droplet of water containing nanoparticles or biomolecules (Fig. 1a). Light passing through it induces an intense evanescent field extending a few hundred nanometres from the fibre surface. Light scattered in the near field of the nanofibre is collected by the guided mode of the fibre, providing a highly localized objective. In contrast to previous approaches, we illuminate a small section of the nanofibre from above with a probe field. Because its propagation direction is orthogonal to the fibre axis, very little of the probe field is collected in the absence of nanoparticles and biomolecules. In this dark-field configuration, minimal background noise is introduced by the probe. When a nanoparticle or biomolecule diffuses into the illuminated region, it scatters probe light by elastic dipole scattering. The nanofibre collects a significant fraction of this field, which we term the ‘signal field’ from here on. Diffusion of the particle in the vicinity of the nanofibre modulates the collection efficiency, encoding information about the motion of the particle on the signal field intensity.

Quantum noise-limited tracking of single unlabelled biomolecules is made difficult by the combination of very low levels of scattered power—in our case in the range of femtowatts—and technical noise sources such as laser frequency fluctuations, laser amplitude fluctuations converted by the apparatus into frequency noise, electronic noise, acoustic vibrations and background scattered light. These technical noise sources are particularly problematic in the hertz to kilohertz frequency band of relevance for observations of biomolecule dynamics, binding and trapping, and are a key limitation of previously reported evanescent sensors. To suppress them, and thereby achieve quantum noise-limited performance, we use an optical heterodyne technique to amplify the signal from the particle above both the electronic noise of our measurement system and noise from background light, and to shift its frequency well away from low-frequency laser, electronic and acoustic noise. In short, an optical local oscillator field frequency shifted by 72.58 MHz from the probe is injected into the nanofibre, and its beat with the signal field is observed on a low-noise photoreceiver (see Methods). A photocurrent proportional to the absolute value of the signal field amplitude is acquired in real time by mixing the photoreceiver output down at the heterodyne beat frequency using a home-built dual-phase lock-in amplifier (Fig. 1).

We performed a sequence of experiments to characterize the noise performance of the biosensor and verify its quantum-limited performance (Fig. 2). These confirmed that electronic noise can be neglected at frequencies above a few hertz (Fig. 2a), and that the local oscillator field is quantum noise-limited (Fig. 2b). The total noise floor of the biosensor was measured while illuminating a static scattering centre, with results displayed in Fig. 2c as a function of collected signal power and equivalent.
particle size at fixed intensity. Quantum noise was found to dominate at all measurement frequencies above 4 Hz, even for the highest signal power used (8 fW, equivalent radius of ~20 nm). This hertz–kilohertz frequency window is important for biophysical processes ranging from seconds to a few milliseconds\(^{18}\), and is the crucial window for measurements of the motion of nanoparticles and biomolecules in solution, as performed here.

The quantum noise limit of our biosensor can be quantified by comparing the shot noise level due to the quantization of light to the scattered intensity predicted from Rayleigh scattering theory (Supplementary Section II.F). The minimum detectable particle cross-section is \(\sigma_{\text{min}} = 8h\omega/\eta I_{\text{probe}}\), where \(h\) is the reduced Planck constant, \(\omega\) is the frequency of the probe light, \(\eta\) is the total collection efficiency including detection inefficiencies, \(\tau\) is the measurement time, and \(I_{\text{probe}}\) is the probe intensity. We estimate the collection efficiency to be in the range of 1–10\%, with more accurate determination precluded by a strong dependence on both the nanofibre geometry and the particle position (Supplementary Section II.B). For our experimental parameters of \(I_{\text{probe}} = 7 \times 10^7\) W m\(^{-2}\) and \(\tau = 10\) ms, the smallest detectable particle cross-section is then predicted to be in the range of \(3 \times 10^{-9}\) to \(3 \times 10^{-4}\) nm\(^2\), corresponding to a silica nanosphere of radius \(20 \pm 4\) nm in water.

The biosensor was tested on solutions of silica and gold nanoparticles in ultrapure double-processed deionized water (Sigma W3500). Unexpectedly, it was able to resolve silica nanospheres of radius down to 5 nm, significantly beneath the quantum noise limit calculated in the previous paragraph. We attribute this to an enhanced scattering cross-section due to the presence of surface charges on the nanoparticles, as discussed in the following.

Figure 3a–c presents sections of typical time-domain traces that display detection events for 25 and 5 nm silica nanospheres and 10 \(\times\) 45 nm gold nanorods, respectively. Calibration of the detected signal in terms of the particle position reveals that the 25 and 25 nm nanoparticles can be tracked with resolutions of 5 and 1 nm, respectively, with a 100 Hz bandwidth (Supplementary Section VI.B). Similar measurements for 15, 35 and 50 nm silica nanospheres are provided in Supplementary Section IV.B, together with a statistical analysis of the detection events. These results compare favourably to other nanofibre sensors, with the smallest nanoparticles previously observed having a 100 nm radius\(^{16}\). Moreover, the system is competitive with the best field-enhanced evanescent sensors both using microcavities\(^{12,4,5}\) and plasmonic resonators\(^{6,7}\), while exposing the specimen to significantly lower optical intensities (as discussed below). This demonstrates the substantial performance gains that can be achieved via dark-field heterodyne detection and complements the recent demonstration of quantum noise-limited super-resolution imaging of stationary proteins\(^{19}\).

The charges that naturally accumulate on surfaces in solution introduce interactions between nanoparticles and the surface of the nanofibre. From each calibrated trace in our experiments it is possible to determine the particle position probability distribution and, using Boltzmann statistics, extract the surface–particle interaction potential (Supplementary Section VI.A). Mean potentials obtained from 5 nm silica nanosphere events are presented in Fig. 3d for a range of salt concentrations. As can be seen, the surface charges introduce a repulsive interaction. This is expected, because silica surfaces become negatively charged in water. The effect of salt is to neutralize this charge and therefore reduce the repulsion, as highlighted in the inset.
means of control experiments and measurements of the contaminants, aggregation and taper vibrations, were excluded by explanations for the enhanced cross-sections, such as outside an absolute value that decreases for salt concentration above surface charge density and the electric double layer (Fig. 3f). Function of salt concentration, as this indirectly characterizes the test, we directly measured the zeta potential of the particles as a section, decreasing the total scattering from them. As a further action as the salt concentration increases, allowing particles to be competed between reduced repulsive particle amplitude for salt concentrations near 25 µM can be explained by competition between reduced repulsive particle–surface interactions as the salt concentration increases, allowing particles to approach closer to the nanofibre, and a reduced scattering cross-section, decreasing the total scattering from them. As a further test, we directly measured the zeta potential of the particles as a function of salt concentration, as this indirectly characterizes the surface charge density and the electric double layer (Fig. 3f). The zeta potential is negative, as expected for silica particles, with an absolute value that decreases for salt concentration above 0.1 mM, consistent with our observation in Fig. 3e. Alternative explanations for the enhanced cross-sections, such as outside contaminants, aggregation and taper vibrations, were excluded by means of control experiments and measurements of the distribution of particle hydrodynamic radii in the sample solution (Supplementary Section III).

Recently, the electric double layer formed by surface charges and their associated counterions has been shown to greatly enhance the scattering cross-section of micelles smaller than 200 nm trapped in an optical tweezer20. Because the surface area-to-volume ratio increases with reduced particle size, a considerably stronger effect could be anticipated in experiments with nanoparticles or nanoscale biomolecules21. The spatial extent of the electric double layer varies strongly with salt concentration due to screening effects. In ultra-pure water, as in our initial experiments, it can extend over several hundred nanometres, whereas it is reduced to the nanometre scale for even relatively modest salt concentrations22.

To test whether an electric double layer enhanced cross section may explain the performance of our biosensor, we explored the effect of salt concentration on the observed detection events for 5 nm particles. As shown in Fig. 3e, the event amplitude and rate are both affected by salt concentration, dropping significantly for concentrations above ~0.1 mM. These observations are consistent with a reduced scattering cross-section, which can be expected to both directly decrease the maximum amplitude and reduce the number of events resolvable above the sensor noise floor. They are also consistent, both qualitatively and in the threshold concentration, with the observations in ref. 20. The peak in the event amplitude for salt concentrations near 25 µM can be explained by competition between reduced repulsive particle–surface interactions as the salt concentration increases, allowing particles to approach closer to the nanofibre, and a reduced scattering cross-section, decreasing the total scattering from them. As a further test, we directly measured the zeta potential of the particles as a function of salt concentration, as this indirectly characterizes the surface charge density and the electric double layer (Fig. 3f). The zeta potential is negative, as expected for silica particles, with an absolute value that decreases for salt concentration above 0.1 mM, consistent with our observation in Fig. 3e. Alternative explanations for the enhanced cross-sections, such as outside contaminants, aggregation and taper vibrations, were excluded by means of control experiments and measurements of the distribution of particle hydrodynamic radii in the sample solution (Supplementary Section III).

With both quantum noise-limited performance and the capability to detect small nanoparticles confirmed, we next applied our biosensor to realize the detection of single unlabelled biomolecules. We performed measurements on very low-concentration solutions of the biomolecules bovine serum albumin (BSA) and anti-\textit{Escherichia coli} (\textit{E. coli}) antibody. Measurements for the larger anti-\textit{E. coli} antibody are provided in Supplementary Section IV.B. Here, we focus on BSA. BSA has a 3.5 nm Stokes radius and is among the smallest molecules detected using plasmonic and micro-cavity-enhanced techniques1,7 (the smallest biomolecules detected so far, to our knowledge, are Syto 62 dye molecules, with ~1 nm radius). Without enhancement, the scattering cross-section of BSA is smaller than the 5 nm nanoparticles detected earlier. Nevertheless, our biosensor is capable of detecting single molecules. A typical detection event is shown in Fig. 4a. We attribute this capability, as before, to an electric double-layer-enhanced scattering cross-section, with aggregation again excluded by measurements of the hydrodynamic radius distribution (Supplementary Section III.A). On measuring the maximum event amplitude as the salt concentration was increased (Fig. 4b), we observed similar behaviour as for 5 nm nanoparticles: the event amplitude initially increases as the repulsive surface–molecule interaction reduces, allowing the BSA to approach closer to the nanofibre, and then decreases very rapidly for salt concentrations above 10 µM as the BSA scattering cross-section decreases.

The ability to detect 3.5 nm biomolecules demonstrates that the performance of our biosensor is competitive in sensitivity with whispering gallery resonators and plasmonic evanescent sensors1,7, although here this is achieved with four orders of magnitude lower light intensity (Supplementary Section VII.A) and a considerably simpler, robust, sensing platform.

Further studies are required to test whether cross-section enhancement due to the presence of an electric double layer might explain previous observations of enhanced sensitivity in evanescent biosensors23, and to explore its wider potential as an enhancement mechanism to improve the detection limits of those sensors. We note that the effect may explain existing observations in the literature2,23,24, such as the very recent and impressive

Figure 2 | Quantum noise-limited region. a, Power spectral density (PSD) of the electronic noise (black) and optical background response (orange) of the system under normal operating conditions. b, Averaged PSD over a 10 kHz bandwidth without probe light as a function of the local oscillator (LO) power (blue points). The linear (orange), quadratic (red) and constant (black) curves represent the quantum model, a classical laser noise model and an electronic noise model, respectively (see Methods). c, Clearance from the quantum noise floor as a function of signal power (left y axis) or its equivalent nanoparticle radius (right y axis) and as function of frequency (x axis) for a stationary scattering source. Signal power is estimated from amplification of the scattered probe field by the local oscillator field (see Methods). The noise floor is dominated by quantum noise in the white-yellow regions, and by technical noise in the orange-black regions.
The evanescent sensor-based observation of single ions in solution, the mechanisms for which remain to be settled.

Specimen photodamage due to exposure to light is often a critical issue in biophysical experiments, resulting in photochemical changes to biological processes, modifying structure and growth, and ultimately adversely affecting viability (Supplementary Section VII.B). For instance, ref. 9 reports that the viability of E. coli is affected by light intensities as low as 4.9 × 10^9 Wm\(^{-2}\). The experiments reported here used probe and local oscillator field intensities of 7 × 10^7 Wm\(^{-2}\).

**Figure 3 | Nanoparticle detection.**
- a-c. Time trace of signal amplitude normalized to the standard deviation of the noise for solutions containing 25 nm (a) and 5 nm (b) silica nanospheres and 10 × 45 nm gold nanorods (c). For clarity, a 4–100 Hz bandpass filter is applied. Shading highlights detection events.
- d. Averaged surface-particle interaction potential as a function of distance from the nanofibre (Supplementary Section VI.A) for 5 nm particles and a range of salt concentrations. The colour of each curve indicates salt concentration: red, ∼0 mM; orange, 1.8 × 10^{-3} mM; yellow, 1.0 × 10^{-2} mM; green, 0.10 mM; light blue, 0.55 mM; dark blue, 5.0 mM. Inset: value of the potential at 10 nm (dashed black line) for each salt concentration. Error bars represent the standard deviation of the value of the potential at 10 nm.
- e. Maximum amplitude (blue, left y axis) of 5 nm nanosphere detection events and their count rate (red, right y axis) for salt concentrations of ∼0, 1.8 × 10^{-3}, 1.0 × 10^{-2}, 0.10, 0.55 and 5.0 mM. The total number of observed detection events for the count rate and amplitude experiments at each concentration were 25, 26, 24, 27, 8, 3 and 9, 10, 9, 11, 6, 2, respectively. Salt: balanced salt solution DPBS (Dulbecco’s phosphate buffered saline, Gibco 14040).
- f. Measured zeta potential of 5 nm particles versus salt concentration. In e and f, curves are guides to the eye, and error bars represent standard error.

**Figure 4 | Biomolecule detection.**
- a. Time trace of normalized amplitude for a typical detection event from a solution containing BSA at 0.26 ng ml\(^{-1}\). The shaded region highlights the detection event.
- b. Mean of maximum amplitude of detection events as a function of salt concentration. The curve is a guide to the eye. Error bars represent standard error. The total number of observed trapping events at each concentration were 12, 8, 9, 7, 10 and 1.
7 × 10^8 W m⁻², below this threshold. Similarly performing plasmonic sensors use intensities of around 1 × 10^13 W m⁻² (ref. 7). More generally, the four orders of magnitude reduction in intensity afforded by our biosensor should allow a commensurate increase in observation time for equivalent photodamage.

The combination of high sensitivity, resolution and bandwidth, together with low photodamage, opens up a new path to explore biophysics in real time and without recourse to ensemble averaging, ranging from cell membrane formation and molecule–molecule interactions to molecular motion and single-molecule dynamics. For example, it may allow single discrete steps in a free flagella motor to be observed at their natural frequencies. So far, such steps have only been observed by attaching a fluorescent label to the flagella and slowing the rotation of the motor by inducing photodamage to reduce the sodium-motive force.

Because our biosensor reaches the quantum noise limit, it can be combined with quantum correlated photons to achieve sub-shot-noise-limited precision. Numerous approaches to quantum-enhanced sensing have been developed over the past few decades. However, as yet they have not been applied to single-molecule sensing. Finally, at the cost of increased intensity, our biosensing approach could be combined with plasmonic sensing by depositing plasmonic particles on the nanofibre. In this way, it may be possible to achieve quantum noise-limited plasmonic sensing and thereby allow the detection of even smaller molecules than is currently possible.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

W.P.B. conceived and led the project. M.A.T. contributed towards the conceptual design. N.P.M. performed the experiments and data analysis, with contributions from L.S.M. Samples were prepared by N.P.M. and M.W. The manuscript was written by N.P.M., W.P.B. and M.A.T. L.S.M. and L.S.M. conceived and led the project. M.A.T. contributed towards the conceptual design. W.P.B. conceived and led the project. M.A.T. contributed towards the conceptual design. W.P.B. conceived and led the project.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. Correspondence and requests for materials should be addressed to W.P.B.

Competing financial interests

The authors declare no competing financial interests.
Methods
Heterodyne concept. The heterodyne strategy is similar to the approach developed in refs 17 and 29 to evade low-frequency noise sources in optical-tweezers-based biological measurements, but, by using heterodyne detection, eliminates the need to phase-stabilize the signal field. To implement the technique, an optical local oscillator field is injected into the nanofibre, frequency-shifted from the probe field by 72.58 MHz. The frequency shift was chosen to be large enough to be free of low-frequency laser noise while remaining within the operating frequency of the acousto-optic modulator (65–95 MHz) and bandwidth of our photodetector. The output of the nanofibre is then detected on a low-noise balanced photoreceiver (see caption of Fig. 1). The interference between the collected signal field and the local oscillator generates a 72.58 MHz beat note in the photoreceiver output, shifted away from technical noise sources and amplified compared to direct detection of the signal field by the factor \( 2 \left( \frac{P_{LO}}{P_{LO}} \right)^{1/2} \sim 10^{-10} \), where \( P_{LO} \) is the power of the local oscillator field and \( P_{Sig} \) is the signal power (Supplementary Section II).

Experimental set-up. Light from a 780 nm diode laser is split into three beams (Fig. 1b). The first beam is frequency-shifted using an acousto-optic modulator (AOM) and used as a probe field. This probe field is focused with a microscope objective onto the nanofibre waist. The second beam, the local oscillator, passes through the nanofibre. Polarization controllers are used in both the probe and local oscillator fields to maximize their interference. After passing through the fibre the combined field is detected on a 800 MHz bandwidth low-noise balanced detector (Newport 1807), with the final beam from the laser used as a balance beam. The photocurrent output from the detector is then passed through a homogenized low-electronic-noise dual-quadrature lock-in amplifier to produce two quadrature signals that are recorded on an oscilloscope. The lock-in amplifier first high-pass filters the photocurrent, then amplifies it, mixes it down using two radiofrequency mixers, and finally low-pass-filters the output signals at 50 kHz. The two mix-downs are performed with a \( \pi/2 \) phase difference to extract the orthogonal quadratures from the heterodyne signal. Each quadrature signal is then digitalized with a Tektronix MDO3054 oscilloscope with a sample rate of 50 kHz or 250 kHz. A final signal proportional to the scattered field amplitude is generated by taking the sum of the squares of the two quadrature signals.

Sample preparation. For each nanoparticle or biomolecule used in our experiments, samples were prepared from manufacturer solution by following a procedure that included shaking, sonication and dilution. The exact preparation process varied for each particle type to ensure that the particles were not aggregated. To test for aggregation, the hydrodynamic radius distribution of particles in each solution was analysed using a Malvern ZS90 Zetasizer. Solutions were used only if the distribution in number was consistent with a quantum noise model due to quantization of the local oscillator power, and inconsistent with the power-squared dependence expected for technical noise. We therefore conclude that the local oscillator field is quantum noise-limited over the frequency range of interest.

Finally, the measurement can be degraded by probe noise. This noise is introduced along with the collected light intensity when the probe scatters from a trapped particle. To characterize it in isolation from the motion of the particle, we aligned the probe to a defect on the surface of the nanofibre. This introduces a stationary source of scattering. The total noise power spectrum of the biosensor can then be characterized as a function of the collected signal power, and calibrated to the quantum noise level via measurements using the local oscillator field alone, as discussed in the main text.

Future improvement in precision. Several avenues exist that may allow further improvements in precision, without increased risk of photodamage. The noise floor could be reduced by a factor of four by replacing the balanced photoreceiver with a single ultralow-noise photodiode, and using homodyne detection and optical phase stabilization, rather than heterodyne detection. Because, in our implementation, the probe intensity was one order of magnitude lower than the local oscillator intensity, further improvements could be obtained by more strongly focusing the probe field, which would increase the scattered photon flux without a significant increase in intensity.

Nanoparticles and biomolecules. The 5 and 25 nm radius silica particles were obtained from Polysciences, the 15, 35 and 50 nm silica particles from Creative diagnostics, and the gold nanorods from Sigma-Aldrich. BSA was purchased from Sigma-Aldrich.

Data availability. The data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request.

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