Biological measurement beyond the quantum limit

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Dynamic biological measurements require low light levels to avoid damaging the specimen. With this constraint on optical power, quantum noise fundamentally limits the measurement sensitivity. This limit can only be surpassed by extracting more information per photon by using quantum correlations. Here, we experimentally demonstrate that the quantum shot noise limit can be overcome for measurements of living systems. Quantum-correlated light with amplitude noise squeezed 75% below the vacuum level is used to perform microrheology experiments within Saccharomyces cerevisiae yeast cells. Naturally occurring lipid granules are tracked in real time as they diffuse through the cytoplasm, and the quantum noise limit is surpassed by 42%. The laser-based microparticle tracking technique used is compatible with non-classical light and is immune to low-frequency noise, leading the way to achieving a broad range of quantum-enhanced measurements in biology.

Laser-based particle tracking enables biological systems to be studied with extremely high precision. When used in conjunction with trapping using optical tweezers, it has allowed the manipulation of viruses and bacteria, unfolding of single RNA molecules, DNA sequencing and the discovery of step-like motion in the biological motor kinesin and muscle protein myosin. The real-time measurement of particle mobility within living cells has revealed information about motor proteins, chemical gradients, protein polymerization, the viscoelasticity of the cytoplasm and viscoelastic changes during cellular processes. The sensitivity of optical tracking experiments such as these is fundamentally limited by noise due to the quantization of light, commonly known as shot noise.

In typical laser-based particle tracking, the presence of a particle causes light to be scattered out of an incident field. The subsequent interference between scattered and transmitted fields manifests itself as a deflection of the incident field proportional to the displacement $x$ of the particle from the beam centre. This deflection is usually detected with a quadrant photodiode. The quantum noise limit is enforced by the probabilistic nature of photon detection events on either side of the photodiode. Quadrant photodetection is a special case of spatial homodyne detection, where information contained in the field mode of interest is extracted via interference with a bright spatially shaped local oscillator field. In this framework, particle position measurement in optical tweezers is formally equivalent to an interferometric phase measurement. It is well known that phase measurements can be improved with non-classical light, with the recent demonstration of sub-shot noise-limited sensitivity in a GEO 600 gravity wave detector being a notable example. The complex scattering pattern from microscopic particles in tracking experiments necessitates a generalization of the usual quantum noise limit for phase measurement. Including spatial structure, and expressed in terms of the position $x$ of the particle, the quantum noise limit is

$$\Delta x_{\text{QNL}}^2 = 1/4 \eta \langle \psi_{\text{det}}(x) \rangle^2,$$

where $\eta$ is the detection efficiency of the scattered light. $\psi_{\text{det}}$ is the mean flux of scattered photons, $\psi_{\text{scat}}$ and $\psi_{\text{det}}$ are, respectively, the mode shapes of the scattered mode and a detection mode defined by the local oscillator field and detection method, and

$$\psi_{\text{scat}} = \frac{d\psi_{\text{scat}}}{dx} \bigg|_{x=0}$$

in the limit of small particle displacement (Supplementary Section S1.1). The quantum noise limit determines the best sensitivity achievable without quantum-correlated light for the given apparatus and photon number, and, although generalized here to include spatial structure, is the benchmark conventionally used in squeezed light experiments. It should not be misconstrued as the more stringent standard quantum limit, which defines a measurement-independent absolute limit to the sensitivity that may in principle be achieved with a given number of uncorrelated photons. Without non-classical correlations, the standard quantum limit can only be reached with a perfect measurement apparatus (Supplementary Sections S1.2–S1.3). Because the quantum noise limit applies to tracking of single particles, it is not constrained by the Rayleigh criterion, which determines the resolvable separation of two particles.

Using an optical field with amplitude quadrature variance $V$ in the detection mode $\psi_{\text{det}}$ in the plane of the particle, the achievable sensitivity is

$$\Delta x_{\text{min}}^2 = [1 - \eta (1 - V)] \times \Delta x_{\text{QNL}}^2$$

(Supplementary Section S1.1). In the coherent state limit with $V = 1$, the quantum noise limit is reached exactly. However, using amplitude squeezed light exhibiting non-classical photon antibunching, the variance $V$ may be suppressed below unity, allowing the

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quantum noise limit to be surpassed. Although equation (1) was derived for squeezed light, when \( V \rightarrow 0 \) it sets an ultimate limit that cannot be surpassed with any quantum resource. When using bright optical fields subject to non-negligible losses, squeezed states approach this ultimate limit and outperform more elaborate non-classical states such as NOON states\(^1\).

Two technical barriers have previously prevented the use of squeezed light in biological measurements or particle tracking. First, such measurements are typically conducted at low frequencies, where classical noise sources constrain the possibility of generating squeezing\(^1\). Second, after propagation through high-numerical-aperture lenses and biological samples, distortion prevents the spatial mode of the squeezed light from matching the detection mode. Here, we have developed a new modality of optical particle tracking to overcome these barriers, shown schematically in Fig. 1. Rather than relying on a single incident field to both interrogate the particle and act as the local oscillator, two separate fields are used. A Gaussian probe field propagates transversely to the optical trapping axis, interrogating the particle and producing scattering, while a ‘flipped’ Gaussian local oscillator field, with a \( \pi \) phase shift applied to one half of its transverse profile, propagates along the trap axis and acts to define the detection mode (see Fig. 2). Direct detection of the interference between the flipped local oscillator and scattered light on a single photodiode provides particle position information equivalent to the quadrant photodiode in standard particle tracking. Now, however, the shape of the local oscillator field can be optimized independently of the probe or trapping fields, and when amplitude squeezed, any spatial mode perturbations occurring during optical propagation are applied equally to both the squeezing and local oscillator, ensuring perfect overlap at detection. Furthermore, the probe field can be stroboscopically pulsed without affecting the local oscillator. This allows a new form of lock-in detection that shifts the particle position information to high frequencies and is compatible with squeezed light\(^2\).

The local oscillator field was generated by an optical parametric amplifier, which, when pumped at 532 nm, produced a 100 \( \mu \)W field with 6 dB of amplitude squeezing. A classical benchmark was produced by removing the pump and adjusting the optical power to match the 100 \( \mu \)W squeezed output, with the quantum noise limit reached at frequencies above 3 MHz. To maximize sensitivity and minimize degradation in the detected squeezing, optical losses were kept to a minimum by the use of 96.5% efficient objectives designed specifically for 1,064 nm light and antireflection coatings on all glass–air boundaries, with the apparatus measured to have a total optical loss of 19%. Including 5% detector loss, the local oscillator was measured with a total efficiency of 76%. However, a higher efficiency of \( \eta = 89\% \) was achieved for the scattered light because it originates within the trap and therefore encounters fewer lossy optical elements (Supplementary Section S2).

The probe field, which illuminates trapped particles from the side, carries a strong amplitude modulation at 3.522 MHz for the stroboscopic position measurement, and a weak phase modulation at 6.5 MHz, which is used to generate an error signal for locking the phase between the probe and local oscillator. Probe photons, which scatter from a trapped particle, then interfere with the shaped local oscillator field. To characterize the stroboscopic measurement system, the probe illuminated a small defect in the sample chamber, producing scattered light to interfere with the local oscillator. The detector output was then studied with a spectrum analyser, which gave the traces shown in Fig. 3a for both squeezed and classical light. The amplitude modulation from the probe is visible as a peak at 3.522 MHz. At this frequency the quantum noise limit is achieved for classical light, while for squeezed light it is surpassed by 2.8 dB, corresponding to a detected squeezed variance of \( V_{\text{det}} = 1 - \eta (1 - V) = 10^{-23}/10 = 52\% \).

**Figure 1 | Experimental layout.** An Nd:YAG laser produces 10–500 mW of 1,064 nm trapping field (orange), which forms a counter-propagating optical trap to immobilize particles. Polarizing optics are used to isolate the trapping field from the detector. An imaging field (green) at 532 nm images the plane of the optical trap onto a charge-coupled device (CCD) camera, allowing particles to be identified visually. A separate Nd:YAG laser produces the 1,064 nm fields of the probe and local oscillator (red), which are used to measure particle position. PBS, polarizing beamsplitter; DM, dichroic mirror; \( \lambda/2 \), half waveplate; AM, amplitude modulator.

**Figure 2 | Schematic of the particle tracking method.** A trapped particle acts as the source of scattered light (faint blue). This scattered light is combined with the spatially antisymmetric local oscillator field (red), collected in an objective, and the interference is measured as intensity fluctuations. The phase of the scattered light is locked such that when the scattering particle is centred, the fields are \( \pi/2 \) out of phase. When the particle moves left, the scattered wavefront shifts closer to the local oscillator field maxima on both the left and right due to the spatial antisymmetry of the local oscillator. This leads to constructive interference. Similarly, moving right leads to destructive interference. Hence, the particle position is encoded on the detected light intensity.

At frequencies lower than 3 MHz, where typical optical particle tracking experiments operate, the noise floor is dominated by technical noise. This noise would preclude reaching the quantum noise limit using a continuous measurement. However, it is avoided with the stroboscopic approach demonstrated here. This allows sub-quantum noise-limited measurements at frequencies down to 10 Hz, matching the lowest frequency previously reported in the literature\(^3\). It is worth noting that at low frequencies technical noise sources such as 1/f noise and laser noise are a common issue in conventional laser-based particle tracking experiments\(^5\). By removing...
this noise, the stroboscopic measurement technique demonstrated here may provide a path to improve these experiments even without using non-classical light.

Initial particle tracking measurements were performed on the trapped thermal motion of 2 μm silica beads in water. The motion is highly overdamped by the surrounding water, with a characteristic Lorentzian mechanical spectrum centred at zero frequency, as observed in Fig. 3b. At high frequencies, the mechanical amplitude scales inversely with frequency, such that motion above 1 kHz is difficult to detect. It is in this high-frequency region that the simplistic model of Brownian motion breaks down, and complex dynamic effects become significant. This section of the observed mechanical spectrum is shown in Fig. 3c both with and without squeezed light. The squeezed light can be clearly seen to improve the sensitivity and also to extend the frequency range over which the mechanical motion is detectable, with the quantum noise limit surpassed by up to 2.7 dB, or 46%. As shown in the inset, the measured squeezing degraded as the trapping power increased, as expected from theory.

These results constitute the first demonstration of quantum-enhanced particle tracking, advancing previous methods used to track the motion of highly reflective mirrors to microscopic and non-paraxial regimes. Furthermore, unlike previous biological experiments with non-classical light, the absolute quantum noise-limited sensitivity is comparable to that in prominent classical experiments, thereby allowing biologically interesting properties to be measured. To demonstrate the scope of the technique, we performed microrheology experiments within Saccharomyces cerevisiae yeast cells. It is known from intracellular measurements with a different yeast strain that the thermal motion of lipid granules in the cell is suppressed by networks of actin filaments within the cytoplasm, causing them to exhibit subdiffusive motion. To study the granule motion in our experiments, the host cell was first immobilized by laser trapping with 170 mW of optical power, which also caused an estimated 1.5 K of cellular heating. The shaped local oscillator was then used to extract lipid granule motion. The sensitivity of the measurement depends on the overlap of the scattered field with the local oscillator, which in our experiment was maximized for small particles near the focus, while the scattering profile from large structures had poor overlap. Because of this, measurements carried out in yeast cells preferentially extracted the motion of lipid granules. Similar to the bead tracking experiments, squeezed light improved the measured sensitivity, with the quantum noise limit surpassed by up to 2.4 dB. In circumstances where optical damage is a concern, this enhancement would allow the probe power to be reduced by 42%.

Figure 3 | Particle tracking spectra. a, Measured noise spectra for a classical (blue) and squeezed (orange) local oscillator without a trapped particle. The small peak visible at 4.7 MHz is caused by the modulations used for locking the laser. To measure mechanical motion, the detected signal was demodulated at 3.522 MHz and recorded with a sample rate of 100 kHz. b, c, A typical measured mechanical spectrum for a 2 μm silica bead closely follows the expected spectrum (light blue, c) for trapped Brownian motion. Squeezed light allows the noise floor on this measurement to be lowered (orange spectra). The inset shows the degradation in squeezing due to increasing trap power with the error bars representing the standard error in the measurements. This agrees well with a theoretical model with no fitting parameters, which assumes that the small fraction (7 × 10−5) of trapping photons that reached the detector contributed shot noise to the measurement.
In measurements of diffusive motion, the key parameter of interest is generally the mean squared displacement (MSD). The MSD of a free particle undergoing thermal motion is

$$\Delta x^2(\tau) = \langle (x(t) - x(t - \tau))^2 \rangle = 2D\alpha^2 \tau$$

where $\tau$ is the delay between measurements, $D$ is the diffusion constant, and $\alpha$ is a diffusive parameter determined by the viscoelasticity of the surrounding medium, with the ratio of loss to storage moduli given by $2^{\alpha} G'/G = \tan(\pi\alpha/2)$. In a purely viscous medium, the particle exhibits Brownian motion, which is characterized by $\alpha = 1$, whereas a viscoelastic medium results in subdiffusive motion with $\alpha < 1$. By measuring $\alpha$, the diffusive regime may be established, and information inferred about the local environment of the particle. Because our particles are confined, this simple theory cannot be applied at long delays where the confinement of the particle introduces a plateau in the MSD.

The MSD was extracted over a range of delay times for both silica beads and yeast results, with typical traces shown in Fig. 4a and b, respectively. The results from silica beads in water match the well-known profile of diffusive motion, with an ensemble of measurements finding that on average $\alpha = 0.994 \pm 0.006$. In contrast, the results extracted with yeast cells reveal clearly subdiffusive motion with a non-stationary value of $\alpha$, which varies on subsecond timescales within the range 0.6–1 as the lipid particles interact with different parts of the local environment (Fig. 4c), similar to measurements reported previously on a different yeast strain. As expected, the MSD observed with squeezed light is similar to that observed with coherent light, but with improved precision. For instance, after a delay of 20 $\mu$s, the particle motion shown in Fig. 4b is resolvable using coherent light but can be resolved using squeezing, with MSDs of $0.8 \pm 1$ nm$^2$ and $1.8 \pm 6$ nm$^2$, respectively. Squeezing was found to allow the diffusive parameter $\alpha$ to be determined with 22% enhanced precision. Equivalently, this allows a 64% increase in the measurement rate while maintaining the same precision. Thus, dynamic changes in $\alpha$ could be observed over shorter timescales, providing more information about the inhomogeneity of the local environment around the granule.

The results reported here demonstrate that squeezed light allows the quantum noise limit on particle tracking to be surpassed within a biological sample. The absolute sensitivity achieved was competitive with previous classical micro rheology experiments; however, the quantum noise limit itself could be lowered if more scattered photons were collected. If 74% more photons were collected, the classical sensitivity would match our quantum enhanced sensitivity. For our setup, this would require increased sample illumination, as even completely eliminating our optical loss would only increase the collection of scattered photons by 12%. Although it is generally easier to increase the light intensity than introduce squeezed light, high light intensities can cause biological damage, so biophysical experiments must operate with constrained optical power. Under such constraints, the enhancement demonstrated provides a way to improve measurement sensitivity without increasing the risk of optical damage to the sample. If the probe intensity were increased even further to equal that used in recent non-biological experiments, the classical sensitivity would approach their demonstrated sensitivity of $1 \times 10^{-28}$ m$^2$ Hz$^{-1}$. By reducing optical loss on the squeezed light to 10%, and using 10 dB of incident squeezing, as has recently been demonstrated in a number of experiments, the quantum enhanced sensitivity could then reach $2 \times 10^{-28}$ m$^2$ Hz$^{-1}$.

Quantum enhanced particle tracking holds increasing relevance, with several experiments approaching quantum limited performance, and a wide range of potential applications. In microrheology experiments, improved sensitivity allows the viscoelastic response of the medium to be probed on smaller timescales, revealing both the properties of the cytoplasm and biological processes at higher frequencies. Several recent experiments have investigated the non-Brownian thermal motion of particles in water on very short timescales, observing hydrodynamic memory and ballistic motion between collisions. However, the instantaneous ballistic motion of a single particle in water remains undetected, as do elastic properties of fluid over very short timescales. Direct observation of the latter without quantum resources would increase the water temperature by over 100 K using 1064 nm light. Further applications include optomechanical experiments in which the quantum state of a trapped levitating particle is measured and controlled; such systems could benefit from enhanced sensitivity both to improve optomechanical cooling and to engineer non-classical states of the trapped particle. More generally, the use of non-classical light could enhance a range of biological measurements techniques, such as two-photon microscopy, super-resolution and absorption imaging.

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Author contributions
M.A.T. and W.P.B. designed the experiment. M.A.T., J.K., J.J., B.H. and V.D. constructed the apparatus. M.A.T., J.J. and V.D. performed the experiments. M.A.T. analysed the data. M.A.T. and W.P.B. wrote the paper, with assistance from all co-authors.

Additional information
Supplementary information is available in the online version of the paper. Reprints and permission information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to W.P.B.

Competing financial interests
The authors declare no competing financial interests.