

Retrodiction of single emitter photon emission rates from nanoscale light emitters in random, dynamic systems

Received: 13 January 2026

Accepted: 14 August 2026

Published online: 25 August 2026

Cite this article as: Lane R., Westerberg N., Hu L. *et al.* Retrodiction of single emitter photon emission rates from nanoscale light emitters in random, dynamic systems. *Sci Rep* (2026). <https://doi.org/10.1038/s41598-026-67530-w>

Richard Lane, Niclas Westerberg, Luna Hu, Eleanor Stride & Daniele Faccio

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

Retrodiction of single emitter photon emission rates from nanoscale light emitters in random, dynamic systems

Richard Lane^{1,+}, Niclas Westerberg^{1,+,*}, Luna Hu², Eleanor Stride², and Daniele Faccio^{1,†}

¹School of Physics and Astronomy, University of Glasgow, Glasgow G12 8QQ, United Kingdom

²Department of Engineering Science, Institute of Biomedical Engineering, University of Oxford, Oxford OX3 7DQ, United Kingdom

*Niclas.Westerberg@glasgow.ac.uk, corresponding author

†Daniele.Faccio@glasgow.ac.uk

+these authors contributed equally to this work

ABSTRACT

Photon emission in biological and chemical systems often consists of large numbers of moving nanoscale emitters with a high spatial density. Estimating the flux of a single emitter within the ensemble is challenging and can be beyond the limit of current imaging techniques. We introduce a generalised quantum retrodiction technique that estimates the single emitter flux from measurements in complex, dynamic systems. We first experimentally validate this using a solution of fluorescent molecules, and then apply it to a cloud of sonoluminescent bubbles in which the single bubble flux is otherwise unattainable. Our approach provides a quantum-informed experimental determination of single emitter photon emission rates in nanophotonic and photoluminescent systems.

Introduction

Direct detection of light from an individual emitter in a randomly distributed collection of identical emitters challenges the limits of standard microscopy. The emission rate and the emission statistics are however, important for a variety of physics from fluorescence lifetime imaging¹ and confocal microscopy² to single molecule spectroscopy³. Here we present a technique for emission estimation that is suitable for such scenarios and beyond. We will focus on two types of emitters with radically different emissive processes: fluorescent molecules and sonoluminescent micro-bubbles.

To give some background, fluorescence correlation spectroscopy (FCS)⁴ is an approach developed to measure spontaneous fluorescence intensity fluctuations of emitters freely moving in solution. The absolute emission rate of the fluorophore (also referred to as brightness)⁵ is however, not directly accessible. This is because FCS is based on computing a normalised autocorrelation function which, by design, discards the individual properties of the emitters. To obtain such information FCS can however, be combined with an analysis of the photon counting histograms^{6,7}. Nonetheless, the proportion of the laser light that leads to excitation of the fluorophore is not known. Indeed, while the number of molecules present in the solution can be reasonably well estimated based on the concentration, this does not yield information about the proportion of molecules that actually fluoresce. Furthermore, FCS can only be used in a certain range of emitter concentrations in order to obtain sufficient signal, and requires a non-microscope based setup if combined with agitations such as heating or stirring of the solvent⁸. As such, this technique serves more as a “rule of thumb” for a particular microscope setup, and is not comparable to a direct measure of the rate of photons emitted from a single molecule. An alternative method is based on localising the molecules and thereby measuring the emission from each individual emitter⁹. This can be done by, for instance, embedding individual molecules in a polymer matrix¹⁰. However, the emission characteristics depend on the local environment^{11–14}, and a polymer matrix is starkly different from a solution.

A somewhat different scenario where photon emission rates are of interest is multi-bubble sonoluminescence (MBSL)¹⁵: the process where multiple bubbles in a liquid collapse and emit light during compression, which can be driven by focused ultrasound. In contrast to fluorophores, the number of bubbles and therefore emitters, constantly fluctuates. Furthermore, the bubble size depend on the dissolved gas concentration as well as the ultrasound frequency and pressure used to excite the bubbles^{16,17}. While bubble clouds have been imaged using high speed videography, resolution is limited due to the conditions: the high oscillation rates, the range of bubble sizes and the high optical scattering^{18,19}. Thus, resolving individual bubbles is currently not possible and the number of emitters involved is not known. However, it has been shown that even micrometre

sized bubbles of perfluorocarbon gases exposed to therapeutic ultrasound generate measurable quantities of light²⁰. In principle, this light could be harvested for drug photo-activation and targeted drug delivery in the human body²¹. There is therefore a need to experimentally detail the emission properties in MBSL. Thus far however, even with counting and time-stamping of individual photons, it has not been possible to measure the number of photons emitted per bubble, per unit time, within a cloud of cavitating bubbles.

In this work, we harness the properties of photon statistics to quantify the emission rates of single emitters in an ensemble. No assumptions about the environment or modelling of the instrumentation are needed. Instead we rely on the sub-wavelength nature of the emitters, and the expectation that they must emit spherical waves either at each event or when averaged over the dynamically varying orientations of the emitter. We count the number of photons detected by imaging optics within a relatively long time period (~ 1 minute). By deconvolving the emission with the drive on (laser for fluorescence, ultrasound horn for MBSL) from that with the drive off, we can retrodict the (statistically averaged) emission only from a single emitter in an ensemble of molecules or bubbles. This retrodiction approach provides a quantum-informed method for estimating the single emitter photon emission rates from nanoscale emitters and can in principle be extended to other sources including e.g. quantum dots and generic fluorescence emitters. We note our method does not provide absolute emission rates of individual, isolated, bubbles, but rather we measure the time-averaged emission rate over a multitude of ultrasound compression cycles which likely involves the destruction and creation of many bubbles in the same location. We will call this an “average” bubble, and each individual bubble will likely behave similar to this. Furthermore, the photons are likely to be emitted in a short burst during each bubble collapse. This does, nonetheless, yield a time-averaged emission rate. Our method treats the bubbles as a steady-state, constant-flux, emitter, and measures this time-averaged emission rate per average bubble. Importantly, this is the rate which is of interest for light-harvesting.

Experimental set-up

Our imaging system, Fig. 1(a), comprises a $4f$ fused-silica aspheric lens $1:1$ optical relay ($f = 50$) coupling a 3D-printed chamber to a photon number resolving (PNR) camera (QCMOS Orca Quest, Hamamatsu). The exposure time was adjusted (20 s – 480 s) to maintain a signal-to-noise ratio (SNR) of > 3 in all frames. We place an adjustable aperture at the Fourier plane of a lens relay system that acts as a low-pass spatial filter. In our experiment, an aperture of 6 mm diameter corresponds to a feature size of $10\ \mu\text{m}$ in the sample plane, so as to ensure that each pixel is (as close as possible) never occupied by more than a single emitter. At a focal length of 5 cm the angular spread of light from an emitter in the Fourier plane is approximately 10 cm. So the iris will block the majority of high frequency spatial modes. We calculate this through the cut-off wavelength of a Fourier-plane pinhole, i.e. $\lambda_c = (d/2\lambda f)^{-1}$ where λ is the wavelength of light, f is the focal length and d is the diameter of the pinhole. It is possible that multiple bubbles could occupy the same pixel volume either within the depth of focus or in the imaging plane. However given that the instantaneous diameter of a single bubble can reach up to $500\ \mu\text{m}$ during volume oscillations, and that larger bubbles are brighter, this is of low likelihood but is a source of uncertainty in the measurement. We also place spectral filters after the aperture and analysed the photon statistics of broadband light by reducing the photon degrees of freedom – polarisation, spatial modes, and spectral modes²².

First, we estimate the emission from fluorescent molecules, Fig. 1(b) in order to benchmark the validity of our technique. We uniformly illuminate a $10\ \mu\text{L}$ volume with a laser power chosen so as to sparsely excite emitters for two different concentrations, 7 nM and 70 nM of a common bright fluorophore, Rhodamine 6G Perchlorate (252441, Sigma Aldrich), diluted in methanol (Uvasol, Merck). To create an equal illumination intensity across all pixels analysed, the excitation beam was shaped into a light sheet perpendicular to the detection plane. This is obtained with a Powell Lens (LGL130, Thorlabs) that converts a collimated input beam with a Gaussian intensity distribution into a vertical laser beam of homogenous intensity. A cylindrical plano-convex lens ($f = 50$ mm, Edmund Optics), and a second rotated cylindrical plano-convex lens ($f = 100$ mm, Edmund Optics) were used to focus and collimate the beam at the centre of the tank. In the second experiment, we used 20 kHz ultrasound to allow bubble clouds to form without the use of very high intensities and/or exogenous nuclei, Fig. 1(c). We inserted a 6 mm sonic dismembrator (FB-120, Fisher Scientific) in a blackout chamber filled with 800 mL of ultrapure water and with an acoustic-absorbing base to prevent ultrasonic reflection (Aptflex F28, Precision Acoustics).

We use the same collection optics and filtering as shown in Fig. 1(a). Furthermore, we used a 35 nm spectral filter centered around 469 nm (FF01-469/35-25, Semrock). We obtained a photon number image by acquiring a frame of 50 s exposure. Note that, due to the highly dynamic nature of the bubble cloud, when integrating over times longer than a few acoustic cycles, the collected light will not be from the same exact single bubble, i.e. our results also rely on the assumption that each bubble in the cloud has similar emission rates.

In both experiments, the exposure times are chosen to match the region in which the QCMOS camera is most sensitive. Theoretically it can measure up to 200 photons/pixel but works best below 30 photons/pixel. This is due to the internal fringe fitting procedure to convert photoelectrons to photon counts.

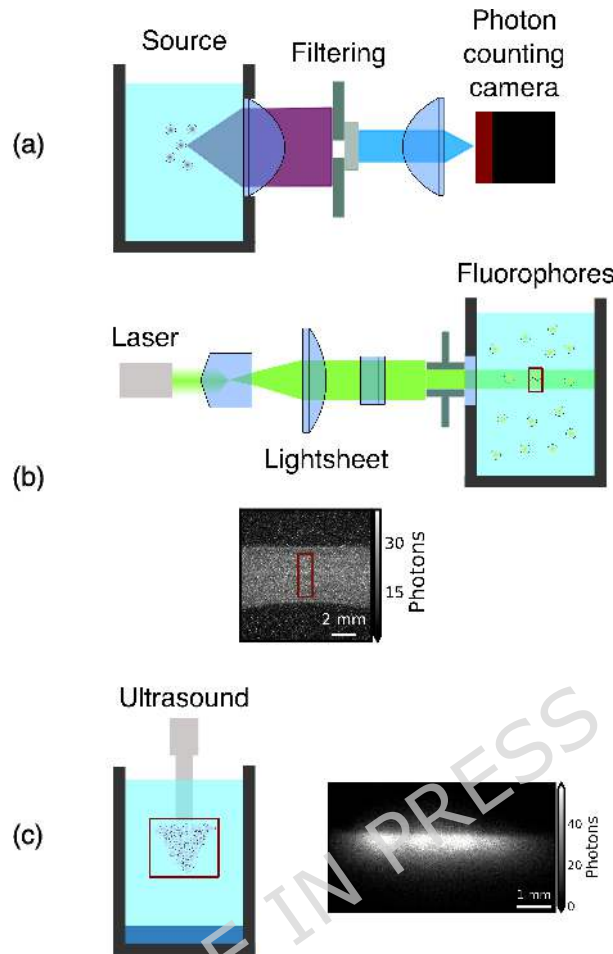


Figure 1. (a) Schematic layout of the imaging system common to both experiments. The emission (here broadband luminescence (purple)) is filtered in the Fourier plane (dark green), and then imaged without magnification (blue) onto $\sim 10 \times 10^5$ pixels of a PNR camera. (b) A triple lens system is used to create laser illumination of even intensity across a macro scale emitter volume; a photon number image of a region of the volume (indicated in red) ($100 \mu\text{m} \times 1.1 \text{ mm} \times 3.4 \text{ mm}$, 6 stacked frames, 7 nM fluorophore concentration, 480 s acquisition time/frame, $0.6 \mu\text{W}$) (c) An ultrasonic horn cavitates a conical volume of microbubbles; an unfiltered photon number image (indicated in red) of the base of a sonoluminescent bubble cone under the tip of an ultrasonic horn (50 s acquisition time).

Theory

Our approach is based on measuring and analysing filtered photoemission from an ensemble of randomly-situated and randomly-oriented emitters. In particular, we focus on the observed photon statistics and analyse its properties, allowing us to extract the single-emitter contribution. Any photon statistics can be characterised in terms of spatial and temporal modes²³. For the case of thermal light, the photon number statistics take the form

$$p(n;M) = \left[\frac{(n+M-1)!}{n!(M-1)!} \right] / \left[\left(1 + \frac{\langle n \rangle}{M} \right)^M \left(1 + \frac{M}{\langle n \rangle} \right)^n \right], \quad (1)$$

where n is the photon number, M is the number of modes and $\langle n \rangle$ is the total average photoemission²³. This is the probability of detecting n photons evenly distributed among M modes for a given average photon number (light intensity) of $\langle n \rangle$. The mode number M can be linked to spatial and temporal coherence, as well as polarisation, and in some circumstances, the number of emitters that illuminate the detection area.

Importantly, we are considering emitters that radiate into a single spatial mode. In the case of fluorescent molecules, these are sub-wavelength by nature and act as electric dipole antennas when absorbing and emitting light. However, the ensemble average will be a uniform spherical wave as the dipole moments of freely diffusing fluorophores are randomly

Figure 2. Applying quantum retrodiction to the emission from fluorescent molecules. **(a)** Photon histogram of total photon counts from 5 sequential images, the average photon number $\langle n \rangle$ is annotated for each image **(b)** Photon histogram of dark counts from 5 sequential images. **(c)** A histogram of the total signal after retrodiction. **(d)** A plot showing the modes measured versus average photon number for 5 sequentially stacked images. The data shown here is the spatially-unfiltered fluorescence data using 7 nM concentration and 22 nW input power. This also serves as an example for the spatially-filtered data, as it has similar character (data not shown).

aligned. Indeed, for emitters (such as fluorophores) that naturally exhibit a dipole emission pattern, the orientation of the emission can nonetheless be treated as random here. This is because the emitter is free to rotate when in solution and we will measure and average over many emission events, as is standard in molecular quantum electrodynamics¹². For sonoluminescent bubbles, ultrasound at kHz frequencies generates an endogenous cloud of several thousand bubbles in the focal zone of the transducer with sizes of order $\sim 10 \mu\text{m}$ and larger. These bubbles then oscillate in the acoustic field and their sizes at the moment of tightest collapse are harder to assess, although typical radii are expected to be of order 100 – 400 nm at the moment of emission²⁴. It has also been suggested that the bubbles emit from an optically opaque core of 100 nm radius, surrounded by an optically transparent halo^{25,26}. Either way, this is sufficiently small as compared to the wavelengths of the emitted photons that the emission will predominately be in a single mode, i.e. a uniform spherical wave, and we have no evidence to suggest that the emission deviates from uniform spherical emission. In fact, anisotropic emission would appear as distinct patterns in the QCMOS images, which can be compared to the image in Fig. 1(c) where the photo-counts simply trace the shape of the ultrasound.

This photoemission from the nanoscale emitters is detected by an array of pixels in a single photon-sensitive (PNR) camera. The low-pass Fourier filter described above is chosen such that each detector (pixel in the camera) measures pre-dominately the incoming on-axis light. This on-axis light and its statistics can then be directly linked to the single-emitter photoemission statistics through the following logic: if we take a single emitter that is located along the axis defined by the detecting pixel, then this will produce photon statistics (as measured by the pixel) with a single spatial mode (two modes, if polarisation is taken into account). Given that each emitter only emits single spatial-mode light, the detected photon rate equals the single emitter emission rate (weighted by the collection and detection efficiencies). Multiple (incoherent and thermal) emitters within the pixel region, which each emit light into a single spatial mode, will create a combined signal with photon statistics of multimode thermal light. By 'thermal light' in this context, we mean that the photon statistics follow the behaviour of chaotic light, an example of which is a thermal light source²³. For example, if we take two nearby and incoherent, thermal emitters which each emit into a single spherical wave (i.e. spatial mode), then these will emit a combined signal that have the photon statistics of two-mode thermal light. We can here take this into account by identifying each spatial mode with an emitter, and extract the number of spatial modes from the statistics. As mentioned however, we wish to limit the number of spatial modes so as to ensure accuracy of the technique – as we show below, the statistical properties of photon emission become less distinguishable at high mode numbers. This is the purpose of the spatial filter in the Fourier plane of the lens, which limits the number of spatial modes while maintaining sufficient signal for reliable statistics. Hence, by photon counting the emission, we can determine the (averaged) number of photons per emission event and per emitter, without any prior knowledge of emitter number or distribution. Also, as already noted, the emission as measured across multiple events and need not be from the same emitter, as

long as the emission is from the same physical space. This is indeed likely the case of multi-bubble sonoluminescence, as well as fluorescent molecules moving freely in a fluid.

In this work, each pixel of the PNR camera is capable of resolving the number of photons accumulated during the measurement time. If we consider a region of pixels, we can gather the required statistics from the distribution of n detected photons over the number of pixels. An example is shown in Fig. 2. Five images are individually histogrammed (a). The exposure times are tailored to the level at which the PNR camera best resolves photons ($n < 30$). Analysis (discussed below) is then performed on each image and the resultant histograms are shown in (c). As a check of the robustness of the technique the mode number is plotted for sequentially stacked images (d). The longer we acquire for the greater the probability that multiple emitters can occupy the same pixel simultaneously so the mode number increases slightly.

Importantly, the raw histogram also contains dark counts, i.e. noise, as shown in (b), obtained by acquiring data under the same conditions as in (a) with the drive off, followed by the same histogramming procedure. We therefore need to separate the noise from the actual photon emission. In other words, we have a measurement of photon statistics and we wish to find the unbiased state which produced this based on any prior knowledge of the experimental set-up. This is a scenario where we can ‘back-propagate’ using Bayes’ theorem in a procedure called quantum retrodiction^{27–30}. The retrodicted photon number probability distribution uses the measured background noise (dark counts) and a given loss rate (see Supplementary Information for details). This retrodicted probability distribution can then be used to extract the average photoemission rate, the effective mode numbers through the variance of the photoemission, as well as other properties.

We find that the unbiased probability that there are m photons present at the source is given by

$$p(m) = \sum_N p(m|N)P(N), \quad (2)$$

where $P(N)$ is the probability that N photons were measured and

$$p(m|N) = \left(\frac{\gamma}{\sum_{n=0}^N P_{\text{dark}}(n)} \right) \sum_{n=0}^N P_{\text{dark}}(N-n) \times \left[\gamma^n (1-\gamma)^{m-n} \binom{m}{n} \theta(m-n) \right] \quad (3)$$

where $P_{\text{dark}}(N)$ is the associated dark count probability. Here γ is the overall efficiency of the optical system, including detection efficiency. The variance of this distribution Δn^2 is connected to the number of modes it contains. Specifically, for thermal light, we have

$$\Delta n^2 = \langle \hat{n} \rangle + \langle \hat{n} \rangle^2 / M. \quad (4)$$

Finally, accounting for the fact that the measured light was emitted into a solid angle that is determined by the optical system, the total emitted light is given by

$$\langle \hat{n} \rangle_{\text{total}} = \langle \hat{n} \rangle_{\text{measured}} / \Delta\Omega, \quad (5)$$

This solid angle is given by

$$\Delta\Omega = \frac{1}{2} \left(1 - \sqrt{1 - \left(\frac{\text{NA}}{\eta} \right)^2} \right), \quad (6)$$

where NA is the numerical aperture of the optical system and η is the refractive index of the medium surrounding the emitters.

Results: fluorescence

To validate our logic, we first provide an estimate of the number of detected photons and emitting molecules from fluorescence. This is a crucial validation step, as we can in this scenario estimate the number of emitters involved, and through this the number of photons that should be detected. We thereby validate the equivalence between the number of spatial modes and number of emitters in these scenarios. As the laser beam propagates through the sample, we expect that the fraction of absorbed photons is $\delta = 1 - \exp(-\sigma\nu L)$, where L is the propagation length, σ is the absorption cross section and ν is the number density. Given an illumination power of P at wavelength λ , this means that $n_{\text{fl}}^{\text{tot}} = \Phi\delta P/\hbar\omega = \Phi\delta P\lambda/(2\pi\hbar c)$ photons/second are expected, where Φ is the fluorescence quantum yield. This is however, distributed over the entire solid angle and we detect only the solid angle portion $\Delta\Omega$ with efficiency γ . Furthermore, we use N_{pixels} of the detector, with ΔL being the detected part of the propagation distance. We also filter both the spectrum and polarisation such that ΔS is the detected, polarised, portion of the fluorescence spectrum. We therefore expect to detect

$$n_{\text{det}} = \gamma\Delta L\Delta\Omega\Delta S n_{\text{fl}}^{\text{tot}} / N_{\text{pixels}} = \frac{\gamma\Delta L\Delta\Omega\Delta S\Phi P\lambda}{2\pi\hbar c N_{\text{pixels}}} (1 - e^{-\sigma\nu L}) \quad (7)$$

Figure 3. Mode number estimation in fluorescence. The effect of changing the power in the laser sheet is shown for two different concentration Rhodamine-6G solutions, 7×10^{-9} M (blue), 7×10^{-8} M (red). The higher concentration (red) rises above the single emitter regime at a lower laser power. Points are marked as single (S) or multiple (M) emitters.

photons/second/pixel. For a molar concentration of 7 nM, we have a molecular number concentration of $\nu = 4.215 \times 10^{12} \text{ cm}^{-3}$. Furthermore, Rhodamine 6G has an absorption cross section of $4.05 \times 10^{-16} \text{ cm}^2$, and a fluorescence quantum yield $\Phi = 0.95$. Given an input power of $P = 22 \text{ nW}$ at 532 nm (6×10^{10} photons/s), total detection efficiency $\gamma = 0.53$ (taking the optical elements into account), solid angle portion $\Delta\Omega = 3.7\%$, and propagation length of $L = 8 \text{ cm}$ and $\Delta L = 249 \times 5 \text{ }\mu\text{m}/L$, we expect $n_{\text{det}} \sim 0.0461$ photons/second/pixel, given that we illuminate $N_{\text{pixel}} = 249 \times 749$ pixels and spectrally filter to $\Delta S \sim 0.5 \times 1/13.35$ of the polarisation (0.5) and fluorescence spectrum ($1/13.35$), respectively. This matches almost exactly with the un-filtered data (see Fig. 2), where we measure 0.0489 photons/second/pixel. This agreement indicates that our experimental setup is indeed collecting the correct number of photons based on known parameters and fluorescent yields of the molecules.

Furthermore, given the number of involved molecules, it is unlikely that any single molecule emits more than once during the acquisition time. We can then estimate that each of the measured emission events (~ 0.0461 photons/second/pixel) originates from a separate molecule. Given 180 seconds acquisition per frame, we would therefore expect to observe around ~ 8.3 emitting molecules per pixel in total. This is largely consistent with the statistics, where we observe ~ 5.7 modes per pixel. This gives us confidence to claim that a spatial mode can be identified with a molecule.

We then apply our method to estimate the emission rate per molecule at two different concentrations. Here we plot the total laser excitation power versus the retrodicted modes in Fig. 3. For both concentrations, the number of ‘retrodicted modes’ (i.e. the numbers of modes, that is molecules, that we predict are contributing to the measured emission) increases as the incident excitation power increases, as is expected due to more molecules being active. We find an emission rate per (statistical) molecule of 0.19 and 0.62 photons/s for the lower and higher concentrations, respectively, in the spectral range of 550 – 555 nm. For the higher concentration, the mode number increases at a faster rate with respect to excitation power when compared to the low concentration solution. In particular, if each mode corresponds to an active molecule, we would expect to see ~ 10 times more modes/unit power in the 70 nM sample as compared to the 7 nM sample. Here we see a ratio of 8.7 of modes/unit power. This is within expectations, as it is difficult to distinguish thermal probability distributions with high mode numbers. For example, a probability distribution with $M = 2$ and $M = 3$ have more differing features than $M = 32$ and $M = 33$. Note that in the limit of $M \rightarrow \infty$, the multimode thermal distribution becomes Poissonian.

In conclusion, we find the expected emission rate from the expected number of fluorescent molecules using our technique. We can, therefore, with confidence link the spatial mode count to the subwavelength emitter count within each pixel, and use this to extract the emission rate per emitter in more general settings, as was the goal. We should highlight that our technique works best at low mode numbers, and thereby low concentration of emitters such as $\sim 10 \text{ nM}$, as otherwise there is both a risk of multiple emitters emitting light from the same (or close enough) spatial location, and hence into the same spatial mode. Also, extracting the mode number with confidence similarly works best at low mode numbers, as the amount of data compared to noise required to confidently extract the mode number increases exponentially (see Supplemental Information). This is simply because thermal photon statistics at high mode numbers (say $M = 31$ and $M = 32$) are very similar to each other. We discuss this further in the Supplementary Information, and this gives us a range of validity to the technique. In our experiments, we find

Figure 4. Quantum retrodiction applied to the emission from sonoluminescent bubbles. **(a)** Photon histogram of total photon counts from 5 sequential images, the average photon number $\langle n \rangle$ is annotated for each image **(b)** Photon histogram of dark counts from 5 sequential images. **(c)** A histogram of the total signal after retrodiction. **(d)** A plot showing the modes measured versus average photon number for 5 sequentially stacked measurements.

that it is advantageous to keep the mode number below 60 for the fluorophores, as can be seen in Fig. 3. In particular, for the 7 nM data, we have SNR of 2.9, 4.5, and 20 in ascending order of laser power. For the 70 nM data, this SNR is instead 59.3, 14.7, and 35, respectively. Very high mode numbers (such as above 60 modes) thus requires a likewise very high SNR. The technique is technically possible to use regardless, but then requires the use of a less noisy environment or brighter emitters.

Results: sonoluminescent bubbles

Figures 4(a) and 4(b) show the collected histograms for the bubble emission and dark counts, respectively. In these measurements, we resolved a total of 2.2×10^6 photons, with a signal-to-noise ratio of 5.93 (estimated as the ratio of the total number of photons detected with the horn on with respect to the total number of dark counts), and total detection efficiency $\gamma = 0.67$. To obtain the average number of photons/s detected per pixel $\langle n \rangle$ we applied a least-squares fit to the maxima of the five histograms Fig. 4(a). In the signal data, $\langle n \rangle$ is 0.14 (total signal counts), and 0.02 (dark counts only). Using both the signal and dark data, the retrodicted probability distributions of finding a pixel that recorded n photons is shown in Fig. 4(c). Following retrodiction, $\langle n \rangle$ decreased to 0.13 (signal), showing that the majority of photons originated from the source.

The number of modes vary slightly, meaning that the retrodicted statistics is not perfectly thermal. This is likely due to crosstalk between the pixels on the photon counting camera, which causes the variance of the observed statistics to scale slightly differently than multimode light, particularly at low photon numbers. As we have no *a priori* reason to expect the light emitted from a cloud of sonoluminescing bubbles to have characteristics that deviate from thermal light, we have to interpret this deviation as the error in our measurement. The mode error is therefore estimated by computing the mode number for the five different integration times (Fig. 4(d)), corresponding to different $\langle n \rangle$ in each image, and considering the variance from the mean mode number.

Therefore, if we analyse the retrodicted photon number probability distributions, we find that 4.0 ± 0.5 modes/pixel were measured in the signal as computed using Eq. (4). Two of these modes originate from the polarisation degree of freedom, so we estimate that we have 2.0 ± 0.25 spatial modes/pixel. Although it is possible to filter the detected field further this would severely reduce the signal, creating a trade-off between the the filtering (which reduces the modes) and the signal-to-noise ratio. We are here at the measurable limit of a single spatial mode. In particular, we find that for the low mode numbers here, an SNR of 3 to 5 is sufficient (tested with synthetic data), but this will increase exponentially with the number of modes (see Supplementary Information).

By dividing the average number of photons/s emitted per pixel by the total number of modes/pixel, we estimate the photon emission rate per single (statistical) bubble to be $1.3 - 1.6$ photons/s in the spectral region of 469 ± 17.5 nm. We should note here that we expect each physical bubble to vary in size and emission characteristics, due to their thermal nature, and as such, this result should be interpreted as the number of photons emitter/s for an *average bubble*, defined such that the total signal is as measured. As an order-of-magnitude estimate, and for comparison with other sonoluminescence experiments, we extrapolate

this to 27 photons/s between 300 – 800 nm (see SM, Fig. S2) by assuming that the emission spectrum is truly a blackbody-like emission profile. In this scenario, the peak emission would be at 132 nm, and we estimate that a total of 83 photons/s/bubble are emitted from the bubble nucleus above the peak wavelength. Note that a peak emission in the extreme ultraviolet is unrealistic, as this would ionise the water as has been noted in literature³¹.

Conclusion

We have demonstrated that we can extract (incoherent) single emitter emission statistics and brightness from a bulk ensemble of emitters. We base this technique on a camera that can detect photon numbers in the low mode and low photon regime. We employ optical Fourier filtering, which isolates the emission from the on-axis emitters and ensures that low mode numbers are detected. This is combined with a retrodictive analysis of the detected photon number statistics, from which we extract the average emission rates as well as confirm that the emission is consistent with single-emitter photon statistics. This retrodictive analysis gives the unbiased probability distribution based on measured probabilities and dark counts, and can be used in any scenario involving an ensemble of incoherent sub-wavelength emitters.

To demonstrate the technique, we studied two such scenarios: laser-excited fluorescence (to validate the technique) and multibubble sonoluminescence. In the case of fluorescence, we first found that the number of spatial modes can faithfully be connected to the number of (incoherent) emitters, when analysing unfiltered data (at very low concentration). We also found that Fourier filtering is required to ensure that the technique is accurate at higher concentrations. When filtered, we found an average emission rate of 0.19 and 0.62 photons/s per molecule in the wavelength band 550 – 555 nm at 7 nM and 70 nM concentrations, respectively. For the sonoluminescent bubbles, on the other hand, we find a photon emission rate for a single (statistical) bubble of 1.3 – 1.6 photons/s, when filtered spectrally around 469 nm.

As this technique is independent of assumptions about the local environment, excitation power density and probability, we foresee that this technique will be useful in scenarios where knowledge about the emission rates, and the photon statistics, is essential.

Funding

The authors wish to acknowledge financial support from the UK Engineering and Physical Sciences Research Council under grant number EP/X033015/1. NW wishes to further acknowledge support from the Royal Commission for the Exhibition of 1851. DF acknowledges support from the Royal Academy of Engineering through the Chairs in Emerging Technologies program.

References

1. Berezin, M. Y. & Achilefu, S. Fluorescence Lifetime Measurements and Biological Imaging. *Chem. reviews* **110**, 2641–2684, DOI: [10.1021/cr900343z](https://doi.org/10.1021/cr900343z) (2010).
2. Tsien, R. Y. & Waggoner, A. Fluorophores for Confocal Microscopy: Photophysics and Photochemistry. In Pawley, J. B. (ed.) *Handbook of Biological Confocal Microscopy*, 169–178, DOI: [10.1007/978-1-4615-7133-9_16](https://doi.org/10.1007/978-1-4615-7133-9_16) (Springer US, Boston, MA, 1990).
3. Plakhotnik, T., Moerner, W. E., Palm, V. & Wild, U. P. Single molecule spectroscopy: Maximum emission rate and saturation intensity. *Opt. Commun.* **114**, 83–88, DOI: [10.1016/0030-4018\(94\)00594-K](https://doi.org/10.1016/0030-4018(94)00594-K) (1995).
4. Elson, E. L. & Magde, D. Fluorescence correlation spectroscopy. I. Conceptual basis and theory. *Biopolymers* **13**, 1–27, DOI: [10.1002/bip.1974.360130102](https://doi.org/10.1002/bip.1974.360130102) (1974).
5. Krichevsky, O. & Bonnet, G. Fluorescence correlation spectroscopy: the technique and its applications. *Reports on Prog. Phys.* **65**, 251, DOI: [10.1088/0034-4885/65/2/203](https://doi.org/10.1088/0034-4885/65/2/203) (2002).
6. Chen, Y., Müller, J. D., So, P. T. C. & Gratton, E. The Photon Counting Histogram in Fluorescence Fluctuation Spectroscopy. *Biophys. J.* **77**, 553–567, DOI: [10.1016/S0006-3495\(99\)76912-2](https://doi.org/10.1016/S0006-3495(99)76912-2) (1999).
7. Yu, L. *et al.* A comprehensive review of fluorescence correlation spectroscopy. *Front. physics* **9**, 644450, DOI: [10.3389/fphy.2021.644450](https://doi.org/10.3389/fphy.2021.644450) (2021).
8. Sahoo, B., Sil, T. B., Karmakar, B. & Garai, K. A Fluorescence Correlation Spectrometer for Measurements in Cuvettes. *Biophys. J.* **115**, 455–466, DOI: [10.1016/j.bpj.2018.05.038](https://doi.org/10.1016/j.bpj.2018.05.038) (2018).
9. Tian, Y., Halle, J., Wojdyr, M., Sahoo, D. & Scheblykin, I. G. Quantitative measurement of fluorescence brightness of single molecules. *Methods Appl. Fluoresc.* **2**, 035003, DOI: [10.1088/2050-6120/2/3/035003](https://doi.org/10.1088/2050-6120/2/3/035003) (2014).

10. Kulzer, F., Xia, T. & Orrit, M. Single Molecules as Optical Nanoprobes for Soft and Complex Matter. *Angewandte Chemie Int. Ed.* **49**, 854–866, DOI: [10.1002/anie.200904858](https://doi.org/10.1002/anie.200904858) (2010).
11. Galbán, J. *et al.* The environmental effect on the fluorescence intensity in solution. an analytical model. *Analyst* **134**, 2286–2292, DOI: [10.1039/B912063G](https://doi.org/10.1039/B912063G) (2009).
12. Craig, D. P. & Thirunamachandran, T. *Molecular quantum electrodynamics: an introduction to radiation-molecule interactions* (Courier Corporation, 1998).
13. Zacharioudaki, D.-E., Fitis, I. & Kotti, M. Review of fluorescence spectroscopy in environmental quality applications. *Molecules* **27**, 4801, DOI: [10.3390/molecules27154801](https://doi.org/10.3390/molecules27154801) (2022).
14. Lyons, A. *et al.* Fluorescence lifetime hong-ou-mandel sensing. *Nat. Commun.* **14**, 8005 (2023).
15. Margulis, M. A. & Margulis, I. M. Contemporary review on nature of sonoluminescence and sonochemical reactions. *Ultrason. Sonochemistry* **9**, 1–10, DOI: [10.1016/S1350-4177\(01\)00096-7](https://doi.org/10.1016/S1350-4177(01)00096-7) (2002).
16. Žnidarčič, A., Mettin, R., Cairós, C. & Dular, M. Attached cavitation at a small diameter ultrasonic horn tip. *Phys. Fluids* **26**, 023304, DOI: [10.1063/1.4866270](https://doi.org/10.1063/1.4866270) (2014).
17. Lee, J., Ashokkumar, M., Kentish, S. & Grieser, F. Determination of the Size Distribution of Sonoluminescence Bubbles in a Pulsed Acoustic Field. *J. Am. Chem. Soc.* **127**, 16810–16811, DOI: [10.1021/ja0566432](https://doi.org/10.1021/ja0566432) (2005).
18. Tan, K. L. & Yeo, S. H. Bubble dynamics and cavitation intensity in milli-scale channels under an ultrasonic horn. *Ultrason. Sonochemistry* **58**, 104666, DOI: [10.1016/j.ultsonch.2019.104666](https://doi.org/10.1016/j.ultsonch.2019.104666) (2019).
19. Yusuf, L., Symes, M. D. & Prentice, P. Characterising the cavitation activity generated by an ultrasonic horn at varying tip-vibration amplitudes. *Ultrason. Sonochemistry* **70**, 105273, DOI: [10.1016/j.ultsonch.2020.105273](https://doi.org/10.1016/j.ultsonch.2020.105273) (2021).
20. Beguin, E. *et al.* Direct Evidence of Multibubble Sonoluminescence Using Therapeutic Ultrasound and Microbubbles. *ACS Appl. Mater. & Interfaces* **11**, 19913–19919, DOI: [10.1021/acsami.9b07084](https://doi.org/10.1021/acsami.9b07084) (2019).
21. Stride, E. *et al.* Microbubble Agents: New Directions. *Ultrasound Medicine & Biol.* **46**, 1326–1343, DOI: [10.1016/j.ultrasmedbio.2020.01.027](https://doi.org/10.1016/j.ultrasmedbio.2020.01.027) (2020).
22. Deng, Y.-H. *et al.* Quantum Interference between Light Sources Separated by 150 Million Kilometers. *Phys. Rev. Lett.* **123**, 080401, DOI: [10.1103/PhysRevLett.123.080401](https://doi.org/10.1103/PhysRevLett.123.080401) (2019).
23. Mandel, L. & Wolf, E. *Optical coherence and quantum optics* (Cambridge university press, 1995).
24. Vazquez, G., Camara, C., Putterman, S. & Weninger, K. Sonoluminescence: Nature’s smallest blackbody. *Opt. Lett.* **26**, 575–577, DOI: [10.1364/OL.26.000575](https://doi.org/10.1364/OL.26.000575) (2001).
25. Dam, J. S. Size of the Light-Emitting Region in a Sonoluminescing Bubble. *Phys. Rev. Lett.* **92**, DOI: [10.1103/PhysRevLett.92.144301](https://doi.org/10.1103/PhysRevLett.92.144301) (2004).
26. Burnett, P. D. S. *et al.* Detailed simulations of sonoluminescence spectra. *J. Phys. B: At. Mol. Opt. Phys.* **34**, L511, DOI: [10.1088/0953-4075/34/16/102](https://doi.org/10.1088/0953-4075/34/16/102) (2001).
27. Barnett, S. M., Phillips, L. S. & Pegg, D. T. Imperfect photodetection as projection onto mixed states. *Opt. communications* **158**, 45–49, DOI: [10.1016/S0030-4018\(98\)00511-2](https://doi.org/10.1016/S0030-4018(98)00511-2) (1998).
28. Barnett, S. M. Quantum retrodiction. In *Quantum Information and Coherence*, 1–30, DOI: [10.1007/978-3-319-04063-9_1](https://doi.org/10.1007/978-3-319-04063-9_1) (Springer, 2014).
29. Barnett, S. M., Jeffers, J. & Pegg, D. T. Quantum retrodiction: Foundations and controversies. *Symmetry* **13**, 586, DOI: [10.3390/sym13040586](https://doi.org/10.3390/sym13040586) (2021).
30. Speirits, F. C., Sonleitner, M. & Barnett, S. M. From retrodiction to Bayesian quantum imaging. *J. Opt.* **19**, 044001, DOI: [10.1088/2040-8986/aa5ccf](https://doi.org/10.1088/2040-8986/aa5ccf) (2017).
31. Ho, C. Y., Yuan, L., Chu, M.-C., Leung, P. T. & Wei, W. Effects of ionization in single-bubble sonoluminescence. *Phys. Rev. E* **65**, 041201, DOI: [10.1103/PhysRevE.65.041201](https://doi.org/10.1103/PhysRevE.65.041201) (2002).

Acknowledgements

The authors also wish to acknowledge insightful discussions with Stephen M. Barnett.

Author contributions statement

RL built the experimental set-up and collected the data. NW devised the theoretical model. LH provided key parts to, and aided in, the experimental set-up. ES and DF conceived the concept and supervised the work. All authors contributed to the analysis and preparation of the manuscript.

Data availability

The datasets used and/or analysed during the current study is available from the corresponding author on reasonable request.

ARTICLE IN PRESS