Computer-based photon-counting lock-in for phase detection at the shot-noise limit

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We implement a simple computer-based photon-counting lock-in that combines the signal-to-noise benefits of photon counting with lock-in detection. We experimentally specify the flatness and the noise characteristics of a flexible software implementation. The noise of amplitude and phase of the small signal is at the limit of photonic shot noise; from 1000 counted photons we reach an amplitude resolution of 4.5% and a phase resolution of 13°. The photon-counting lock-in reduces illumination noise, detector dark count noise, and can suppress background. In particular, phase detection is useful to image the delay characteristics in microscopic systems by use of fluorescent probes that are designed to report membrane potential, temperature, or concentration in a chemical reaction.

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Recently, there has been great interest in sensitive biofluorescence from a small number of molecules. Since the fluorescent probes eventually bleach, photon-counting techniques have been used regularly to collect fluorescence with maximal sensitivity. Light detection can be further improved by use of the lock-in principle. We implemented a computer-based digital dual-phase lock-in amplifier by use of transistor–transistor logic- (TTL-) based photon-counting detectors to measure the relative small-signal amplitude and the small-signal phase at the shot-noise limit.

Lock-in amplifiers are widely used to maximize the signal-to-noise ratio of analog signals. A lock-in detects a signal at a known modulation frequency in amplitude and phase and suppresses noise at other frequencies. The lock-in detection principle can enhance the signal-to-noise ratio by orders of magnitude. Furthermore it can detect important delay information by use of the phase signal. Analog implementations of lock-in detection are applied in frequency-domain fluorescence lifetime measurements[1] and have been used to separate four dyes of different lifetimes by use of two modulated illuminations.[2]

However, light detection with analog detectors is inferior to single-photon-counting detectors, especially at low light levels.[3,4] Photon-counting detectors produce single-photon pulses by implementation of mechanisms of high internal gain. The detector performance is then unaffected by the noise of subsequent electronic circuits. The lock-in principle was first applied to photon-counting detectors by Arecchi *et al.*[5] and was used subsequently in many low-light measurements.[6] A dual-phase implementation of the above gated photon counting is hampered by signal pick up from harmonics under nonsinusoidal modulation. To obtain a precise phase signal, TTL photon counts were reconverted to analog signals that feed into an analog lock-in.[7] Care must be taken to prevent noise pick up and phase distortions. The analog conversion also makes it difficult for one to measure a relative small-signal amplitude by normalizing it against the dc background. This is essential for fluorescence measurements but is not provided by analog lock-in amplifiers. The described computer-based photon-counting lock-in does not have the above-mentioned drawbacks and directly measures both the relative small-signal amplitude and the phase from a photon-counting detector. We show experimentally that both amplitude and phase reach the fundamental noise limit of photonic shot noise.

Preliminary results suggest that the photon-counting lock-in is particularly useful for biological fluorescence detection. Fluorescent molecules have been developed with sensitivity to properties such as membrane potential, temperature, or chemical reactions. We can utilize the lock-in discrimination by modulating the property to which the dye is sensitive under constant illumination. We thus image the systems's response at high sensitivity with molecular resolution in amplitude and phase. The power of this lock-in approach was demonstrated for an analog lock-in implementation to measure the membrane potential of stimulated neurons.[8,9] The analog lock-in enhanced the detection resolution by more than an order of magnitude. Our ongoing experiments use the superior sensitive approach of the photon-counting lock-in to detect opening and closing kinetics of molecular beacons[10] to enhance the detection of DNA.

We chose a software approach, extending the methods of digital lock-in amplifiers[11] (Fig. 1). The source

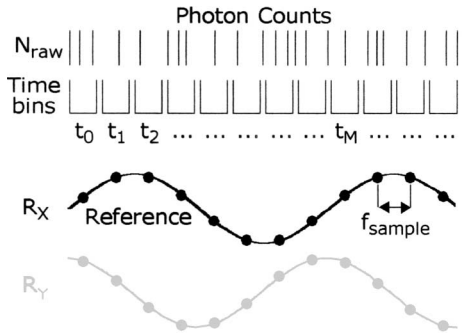

Fig. 1. Implementation of the photon-counting lock-in. Projections of time-binned counts N_{raw} to sinusoidal reference R_X and cosinusoidal reference R_Y are time averaged to yield the small I_X and I_Y signals of the lock-in.

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code (LabView, National Instruments, subroutines in C) can be obtained from the authors.[12] We record photon counts $N_{\mathrm{raw}}(t_n)$ in time bins t_n at a sample frequency f_{sample}. The sample frequency oversamples the reference frequency with $f_{\mathrm{sample}} = M^* f_{\mathrm{ref}}$. Typically we used an oversampling of $M = 50$. We time locked the synchronously buffered TTL counting (NI-6602, National Instruments, Tex.) to an analog-to-digital (AD) converter (PCI-MIO-16E-1, National Instruments) by connecting the SCANCLK signal of the AD card to the gate of the counter. The AD converter digitizes the sinusoidal or rectangular reference signal $R_{\mathrm{raw}}(t_n)$. For flexibility, both cards are not locked to the reference frequency. Instead, we used an algorithmic phase-locked loop as follows. We subtracted time average $\langle R_{\mathrm{raw}} \rangle$ from R_{raw} defined by

$$\langle x \rangle = \frac{1}{N} \sum_{n=0}^{N-1} x(t_n) . \tag{1}$$

We interpolated the zeros with positive slope given at times t_z. A linear regression of t_z versus z yields the reference frequency f_{ref} as the slope and the reference phase φ_{ref} as the y-axis segment. Both are the basis for computation of a sinusoidal X reference $R_X(t_n)$ and a cosinusoidal Y reference $R_Y(t_n)$ at time points t_n with frequency f_{ref}, phase φ_{ref}, and an arbitrary amplitude (Fig. 1). We subtracted the time average $I = \langle N_{\mathrm{raw}} \rangle$ from the photon counts $N_{\mathrm{raw}}(t_n)$ to find $N(t_n)$. These photon counts $N(t_n)$ are then projected to the functional basis of sine R_X and cosine R_Y to obtain the complex-valued relative small signal $\Delta I/I$:

$$\frac{\Delta I}{I} = \frac{I_x + i I_y}{I} = \frac{\sqrt{2}}{\langle N_{\mathrm{raw}} \rangle} \left(\frac{\langle N R_x \rangle}{\sqrt{\langle R_x{}^2 \rangle}} + i \frac{\langle N R_y \rangle}{\sqrt{\langle R_y{}^2 \rangle}} \right) . \tag{2}$$

Usually we plot the small signal as the relative small-signal amplitude $A = |\Delta I/I|$ and small-signal phase $\varphi = \arctan(I_Y/I_X)$. Even at $f_{\mathrm{sample}} = 500$ kHz and $f_{\mathrm{ref}} = 10$ kHz, computation time does not exceed the measurement time on a modern computer system.

From the same photon counts we can obtain N_{raw} periodically averaged phase-locked transients as used in a previous analog implementation.[8] Instead of a sinusoidal reference R_X, we now calculate a sawtooth-shaped, periodic index table $m(t_n)$ at frequency f_{ref} and phase ϕ_{ref} with values of m between 0 and $M - 1$. From this we obtained the averaged relative small-signal transients $\Delta I_k/I$ within a reference period using the time index $k = 0 \ldots M - 1$:

$$\frac{\Delta I_k}{I} = \frac{\sum_{m(t_n)=k} N(t_n)}{\langle N_{\mathrm{raw}} \rangle \sum_{m(t_n)=k} 1} . \tag{3}$$

We measured the flatness of the photon-counting lock-in over frequency by monitoring a sinusoidally modulated light-emitting diode (LED) at $f_{\mathrm{ref}} = 1$ Hz–10 kHz with $f_{\mathrm{sample}} = 50^* f_{\mathrm{ref}}$. The applied ac voltage has an amplitude of 60 mV (33120A, Agilent) at an offset of 2.2 V (E3640A, Agilent), which yields a relative light intensity amplitude of 20% as inferred from the dc characteristic of the LED. We recorded the light with a photon-counting photomultiplier (P10PC, Electron Tubes) in a microscope setup (10×0.3 NA, Zeiss Axioscope). Within 2-s measurement time, we counted 2×10^5 photons, including 48 dark counts. Amplitude and phase computed with the photon-counting lock-in show a flat characteristic over frequency as expected (Fig. 2).

The ultimate noise limit of single-photon detection is shot noise. We assess the noise performance of the photon-counting lock-in to prove that amplitude A and phase φ reach the limit of photonic shot noise. We use the above setup conditions and reduce the illumination of the photomultiplier to decrease the number of counted photons by changing the size of an aperture in the image plane in front of the photomultiplier. We plot the standard deviation σ of the counted photons $I = \langle N_{\mathrm{raw}} \rangle$, relative amplitude A, and phase φ, determined from 40 measurements, against the number of counted photons I of a single measurement with a recording time of 1 s (Fig. 3). We find that the measurement errors given by the standard deviation match the photon shot-noise limit:

$$\sigma(I)/I = 100\%/\sqrt{\langle N_{\mathrm{raw}} \rangle} , \tag{4}$$

$$\sigma(A) = 100\%/\sqrt{\langle N_{\mathrm{raw}} \rangle} , \quad \sigma(\varphi) = 360^\circ/\sqrt{\langle N_{\mathrm{raw}} \rangle} . \tag{5}$$

In photon counting without locking, we can measure only photon counts I at the noise limit [Eq. (4)]. When we use the photon-counting lock-in, we can also detect the small-signal amplitude and small-signal phase at the same fundamental detection limit [Eqs. (5)] as shown in Fig. 3.

In biological applications, for example, we can count approximately $1 = 10^4$ emission photons from a single fluorescent dye before it is bleached. We can thus detect a phase error down to 3.6° from a single molecule

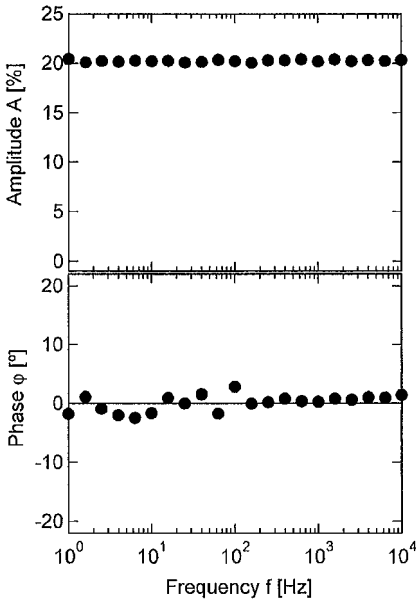

Fig. 2. Flatness of the photon-counting lock-in checked from 1 Hz to 10 kHz with a modulated LED. Each point is the lock-in output from 2×10^5 counted photons within 2-s measurement time.

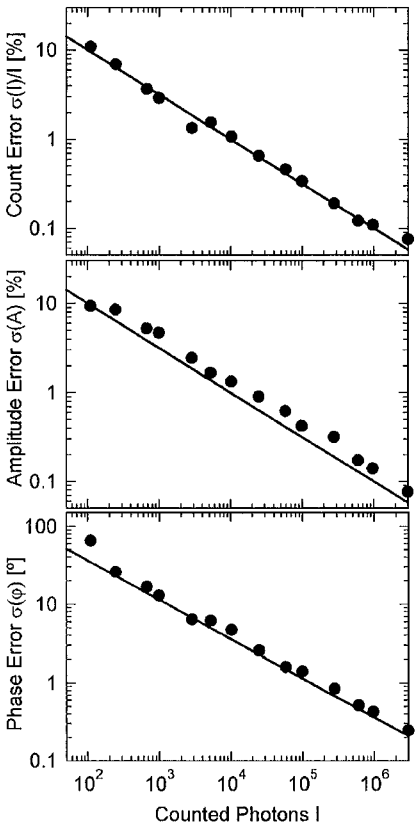

Fig. 3. Noise performance. The photon-counting lock-in operates at the shot-noise limit of photon counting. The relative error of photon counts I, relative amplitude A, and phase φ is inversely proportional to the square root of the number of counted photons I as given by Eqs. (4) and (5).

[Eqs. (5)]. Note that the above error limits are independent of the modulation frequency used. Even in rather slow schemes such as temperature modulation[10] with modulation frequencies of $f_{\mathrm{ref}} = 10\,\mathrm{kHz}$, the above phase error corresponds to 1 μs, which is faster than most biologically relevant reactions. Reaction dynamics could therefore be measured from one single molecule, surpassing methods that must be averaged over many single-molecule properties such as fluorescence correlation spectroscopy.

We have implemented a simple computer-based lock-in for TTL-based photon-counting detectors as a flexible software implementation[12] by using low-cost acquisition cards. We have demonstrated flatness up to 10 kHz and have shown that relative amplitude and phase are detected at the limit of photonic shot noise. The photon counting enabled us to reduce noise in low-light measurements and allowed us to measure delay information from the phase signal in important biological applications.

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