Setting bounds on two-photon absorption cross-sections in common fluorophores with entangled photon pair excitation

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Excitation with entangled photon pairs may lead to an increase in the efficiency of two-photon absorption at low photon flux. The corresponding process, entangled two-photon absorption (E2PA), has been investigated in numerous theoretical and experimental studies. However, significant ambiguity and inconsistency remain in the literature about the absolute values of E2PA cross-sections. Here, we experimentally determine upper bounds on the cross-sections for six fluorophores that are up to four orders of magnitude lower than the smallest published cross-section. For two samples that have been studied by others, Rhodamine 6G and 9R-S, we measure upper bounds nearly four and five orders of magnitude lower than the previously reported cross-sections.

I. INTRODUCTION

Two-photon excitation microscopy is a widely used technique for cellular imaging deep within biological tissues. It relies on two-photon absorption (2PA) in a fluorescent molecule, where a nearly simultaneous absorption of two photons leads to a transition from the ground state to an excited state and subsequent fluorescence. Under coherent laser excitation, 2PA is an incredibly unlikely process. To increase the probability of 2PA, light is typically concentrated into short optical pulses and focused to a small spot size, ensuring the photons are well overlapped in time and space [1,2]. Nevertheless, most of the incident photons are not involved in 2PA. In biological samples, many of these extra photons instead lead to heating and other forms of damage, which can disrupt biological processes [3].

These practical concerns, along with fundamental interest in quantum metrology and spectroscopy, have stimulated theoretical and experimental studies investigating the possibility of enhancing the efficiency of 2PA by exciting with nonclassical light. Photon pairs that are entangled in the energy-time and position-momentum degrees of freedom can exhibit the strong temporal and spatial correlations needed for 2PA. Theoretical studies on simple model systems [4-6] have predicted that using entangled photon pairs can lead to a significant quantum advantage in 2PA rates. Here we define quantum advantage as the ratio of minimum photon flux necessary to observe classical 2PA (C2PA) to that for entangled 2PA (E2PA).

A number of experimental studies have investigated E2PA and concluded that a large quantum advantage does indeed exist [7-14]. In one case, E2PA was reportedly measured at 10 orders-of-magnitude lower excitation flux than that required to observe C2PA [10]. However, in many of these reports it is unclear whether the signals are caused by E2PA or some other process. For example, some of the reports from Goodson and coworkers [7-13,15] and Villabona-Monsalve et al. [14] conclude that the signals they observe are E2PA based only on its linear dependence on photon flux. However, in some cases, the trend of the data and the magnitude of the measurement uncertainty does not exclude a nonlinear fit. Furthermore, a linear dependence is consistent with many one-photon processes such as scattering, one-photon absorption (1PA), or fluorescence from the coating of an optic in the beam path.

Another signature of E2PA is the signal’s dependence on the time delay between photons in a pair; as the time delay is scanned away from optimal overlap (zero time delay), the signal is expected to decrease towards zero, in accordance with the simultaneity requirement of 2PA. In a recent report, Tabakaev et al. [15] observed such behavior in an entangled two-photon excited fluorescence (E2PEF) experiment. An interferometer was used before the sample to probabilistically and equally split photons and time delay half of the photon pairs, while the other half traversed the same path. The resulting E2PEF as a function of the time delay should consist of a constant signal due to the photon pairs that traveled the same path and a variable signal due to the photon pairs that traveled different paths. The signal at long time delays should be half the signal at zero delay. Instead, Tabakaev et al. observed a signal that tended to zero at large time delays. This unexpected result was pointed out by the authors of the study, but it was not resolved. Some of the other reports [7-9,16] have included time-delay scans, but with data reported at only a few delays. Recently [14], we detailed the difficulty in identifying E2PA signals through transmittance-based schemes with and without implementing a time delay. Based on previously reported cross-sections [8,14], we found that the mag-
nitude of the expected change in transmittance due to E2PA for the molecule Zinc tetraphenylporphyrin is of similar magnitude to the change in transmittance due to delay-dependent alignment shifts and residual interference. These factors can lead to the misinterpretation of signals measured with or without time delay techniques. In all of the aforementioned reports by other groups, the E2PA detection sensitivity—and thus the measurement confidence level—was left unspecified. These ambiguities leave unanswered questions regarding the magnitude of the quantum advantage.

Although the tantalizing prospect of a large quantum advantage remains, experimental methodology has not yet evolved to a point where meaningful comparisons between experiments by different groups is straightforward, where interpretation in theoretical context is possible, or even where the relative magnitudes of the C2PA and E2PA signals can be measured in the same experiment. Our study exposes and addresses some of these issues. We emphasize the role of the spatio-temporal correlations of the excitation source on E2PA and the importance of providing these characteristics when reporting the absolute cross-section values.

To directly address the question of the quantum advantage, we present a method for measuring both E2PEF and classical two-photon excited fluorescence (C2PEF) in one experimental setup. The sensitivity of our experiment is quantified using C2PEF, one-photon excited fluorescence (1PEF), numerical simulations and calculations. The C2PEF measurements probe six fluorophores with previously reported values of the C2PA cross-section, \( \sigma \) [13, 18, 19]. Although we do not detect measurable E2PEF signals for any of the six fluorophores, we can bound the maximum efficiency of the E2PA process in each fluorophore by placing upper bounds on its E2PA cross-section, \( \sigma_E \) (defined more precisely in Sec. II), based on our E2PA detection sensitivity. These upper bounds are used with the C2PEF measurements to also bound the maximum possible quantum advantage. The established upper bounds on \( \sigma_E \) are up to four orders of magnitude lower than the smallest published value of \( \sigma_E \) [13]. For two of the samples, the upper bounds on \( \sigma_E \) are nearly four and five orders of magnitude lower than previously reported [13] [15].

In Section II, we provide a simple theoretical description for the rate of E2PA and the corresponding cross-section. Afterwards, we discuss our experimental setup and the characterization of the entangled photon source and fluorescence collection unit in Section III. We present our results—including the upper bounds on the E2PA cross-section and quantum advantage—in Section IV. Further details about the experiment and analysis are given in the appendices.

II. THEORETICAL BACKGROUND

The rates of multi-photon processes are sensitive to the photon statistics [20, 21], which can be characterized by the second-order coherence, \( g^{(2)} = \langle \hat{a} \hat{a}^\dagger \hat{a}^\dagger \hat{a} \rangle / \langle \hat{a}^\dagger \hat{a} \rangle^2 \), where \( \hat{a}^\dagger \) and \( \hat{a} \) are the photon creation and annihilation operators. For a single-mode field with mean photon number \( \mu = \langle \hat{a}^\dagger \hat{a} \rangle \), the 2PA rate can be written [22, 23]

\[
R = \kappa_2 \left( \langle \hat{a}^\dagger^2 \hat{a}^\dagger \hat{a}^\dagger \hat{a} \rangle \right) = \kappa_2 \mu^2 g^{(2)},
\]

where \( \kappa_2 \) is a collection of constants quantifying the strength of the nonlinear interaction. It has been demonstrated, for example, that thermal light \( g^{(2)} = 2 \) doubles the 2PA rate compared to laser (coherent) excitation \( g^{(2)} = 1 \) of the same intensity [24].

For pulsed laser excitation of a two-photon-absorbing molecular system, \( g^{(2)} = 1 \) and

\[
\kappa_2 = \frac{\sigma_C}{2T^2A^2},
\]

where \( T \) and \( A \) are the pulse duration and beam area, and the C2PA cross section \( \sigma_C \) has units of GM (1GM = 10^{-50} \text{ cm}^4 \text{s} ). Rewriting Eq. (1) in terms of photon flux \( \phi = \mu/TA \) leads to the familiar phenomenological expression for the C2PA rate [25]:

\[
R = \frac{1}{2} \sigma_C \phi^2.
\]

In contrast to laser light, spontaneous parametric downconversion (SPDC) produces photon pairs exhibiting correlations in energy, time and space that can be tailored to enhance the rate and selectivity of 2PA [26, 27]. The energy correlations between the signal and idler photons within a pair are set by conservation of energy in the conversion of one pump photon to two down-converted photons and can be engineered to match the energy of a two-photon transition. Photon pair production is localized in space and time [28, 29], allowing for excitation with photons that nearly simultaneously arrive in a localized region of space.

A degenerate, single-mode [30] photon pair generated by SPDC can be modeled as a single-mode squeezed vacuum, for which \( g^{(2)} = 3 + 1/\mu \) [22]. Substituting this expression into Eq. (1) yields

\[
R = \kappa_2 (\mu + 3\mu^2).
\]

For a single-mode pulsed source, substituting Eq. (2) gives

\[
R = \frac{1}{2} \sigma_C \left( \frac{\phi}{TA} + 3\phi^2 \right).
\]

An alternative way to write the E2PA rate is [6]

\[
R_E = \frac{1}{2} (\sigma_E \phi + 3\sigma_C \phi^2),
\]

in which \( \sigma_E \) and \( \sigma_C \) are the E2PA and C2PA cross-sections, respectively.
where the E2PA cross-section has units of cm$^2$. For a single-mode field, the two cross-sections are related by $\sigma_E = \sigma_C/T A_e$. At low excitation flux, the first term dominates and the E2PA process should scale linearly with $\phi$. At a large photon flux, where many photon pairs overlap in time, the quadratic term will dominate.

In a real experiment, the SPDC light will typically occupy multiple modes and Eq. (4) will not hold. In this case, the coefficient for the linear term could in principle be larger than the coefficient for the quadratic term. Roughly speaking, if the two photons in a pair are more closely correlated in time than the pump pulse duration, the interaction strength could be enhanced by the factor $T/T_e$, where $T_e$ is the entanglement time. Analogously, if the photons in a pair are more closely correlated in space than the beam size, the interaction strength could be modified by the factor $A/A_e$, where $A_e$ is the entanglement area. The values of $T_e$ and $A_e$ are set by the temporal and spatial $g^{(2)}$ functions and illustrated in Fig. 1. Following this argument leads to the approximation

$$\sigma_E \approx \frac{\sigma_C}{T_e A_e}. \quad (7)$$

To maximize the E2PA rate, $T_e$ and $A_e$ should be as small as possible. For a large $\mu$ and a large number of modes, $g^{(2)} \to 1$ and the E2PA excitation rate approaches the classical limit in Eq. (3).

Unlike $\sigma_C$, which depends only on wavelength for a particular molecular 2PA transition, the value of $\sigma_E$ depends strongly on the properties of the excitation source and experiment. The values of $A_e$ and $T_e$ evolve as the SPDC beam propagates through optics from the down-conversion crystal to the sample, and therefore will depend on the details of the optical system used to measure E2PA. Calculating $\sigma_E$ for a given experimental geometry thus requires knowledge of $A_e$ and $T_e$ within the excitation volume. Clearly, these factors complicate the ability to compare results from different experiments.

A summary of important parameters in selected E2PA reports is given in Table I. These studies are among the most complete experimental efforts from several independent groups, which we can use for comparison here. Table I shows $\sigma_C$ and $\sigma_E$ values determined at several near infrared wavelengths. In Ref. [13], pulsed-pumped type-II SPDC was generated to excite a sample with $1 - 25 \times 10^6$ photons s$^{-1}$ in fluorescence- and transmittance-based E2PA schemes. Results obtained with these two techniques produced a spread in the reported $\sigma_E$ values for the studied 9R-S molecule. The measurement uncertainty was estimated to be 9% and 12% for transmittance- and fluorescence-based techniques. In Ref. [15], a continuous-wave-pumped type-0 SPDC source was used for E2PEF measurements with an incident photon rate of $2 - 50 \times 10^7$ photons s$^{-1}$ (beam waist of 60 $\mu$m). An increase from the micromolar to the millimolar level of concentrations led to a decrease in the measured $\sigma_E$ value for Rhodamine 6G (Rh6G) by more than a factor of two. The uncertainty on the measured cross-sections were estimated to be nearly 50%. A similar concentration dependence was observed in Ref. [14] using pulsed-pumped type-II SPDC excitation with an incident pair rate of $50 - 7,000$ photon pairs s$^{-1}$ (beam waist of 61 $\mu$m) in a transmittance-based scheme, where the concentration dependence of $\sigma_E$ for Rhodamine B (RhB) was attributed to potential aggregation effects in used solutions. The uncertainty on the published cross-sections are $\approx 10\%$. In Ref. [11], a pulsed-pumped type-II SPDC source was used to excite the tetraannulene sample with $1 - 25 \times 10^6$ photons s$^{-1}$ in a transmittance-based E2PA scheme. The measurement uncertainty was not estimated in this report. The value of $\sigma_E$ of $990 \times 10^{-19}$ cm$^2$ for tetraannulene and 0.0099 $\times 10^{-19}$ cm$^2$ for Rh6G are the largest and smallest values, respectively, that have been reported. In all of these reports the photon flux was not specified, except for an order-of-magnitude estimate in Ref. [14], and the photon rate was not precisely defined.

No direct $T_e$ measurements were completed in the aforementioned reports. In the case of Ref. [15] we estimate $T_e$ based on the details provided by the authors, who estimate an effective flux reduced to the fraction of photon pairs that have $T_e = 140$ fs. In Refs. [11] [13] [14], the value of $T_e$ is estimated at the output of the crystal, which in some cases can be orders of magnitude smaller than the value at the sample’s position. This is especially true when the total group delay dispersion (GDD) of the optics is large, as in Ref. [11], or when the bandwidth of SPDC is large, as in Ref. [11]. The value of $T_e$ would be very sensitive to even small amounts of GDD in the later case. The values of $A_e$ were not specified, however we use Eq. (7) to estimate the entanglement area, $A_e^{\text{ext}}$, of the source used in each publication. Assuming Eq. (7) holds, in all of the reports $A_e^{\text{ext}}$ ranges from $10^{-6} - 10^{-7} \mu$m$^2$. This would require both photons within a pair to be con-
TABLE I. Results and experimental parameters from selected E2PA studies. Cross-sections ($\sigma_C$ and $\sigma_E$) are quoted at the corresponding excitation wavelength ($\lambda$). Cross-sections and entanglement times ($T_e$) are taken directly from the reports unless otherwise noted. We estimate entanglement area ($A_{ent}^{est}$) based on Eq. [\ref{eq:ent_area}].

| Sample [Ref.] | $\lambda$ (nm) | $\sigma_C$ (GM) | $\sigma_E$ (10$^{-19}$ cm$^2$) | $T_e$ (fs) | $A_{ent}^{est}$ (10$^{-9}$ $\mu$m$^2$) |
|---------------|----------------|----------------|-----------------------------|------------|-------------------------------------|
| Rh6G [15]    | 806            | 28             | 2.02-2.69                   | 100        | 1.0-1.4                             |
| RhB [14]     | 808            | 260 $\pm$ 40   | 0.0009-0.019                | 14         | 38-72                               |
| Tetraannulene [11] | 800            | 2960           | 0.17-42                     | 17         | 3.6-900                             |

$^a$ From Ref. [36]; $^b$ Not explicitly written in report, but we estimated based on reported details

fined to a region that is a factor of $10^{-6} - 10^{-10}$ smaller than the diffraction limited spot size. We have no evidence that this level of confinement is feasible. Thus, the theory \cite{6} that derived Eq. \eqref{eq:ent_area} cannot explain these experimental results, but theoretical work on this topic is ongoing \cite{37}.

III. EXPERIMENTAL SETUP AND CHARACTERIZATION

Here we give a brief overview of our experimental setup and characterizations. A thorough description of the components is given in Appendix A. A schematic of the experimental setup is shown in Fig. 2. A pump laser emits $\approx$ 110 fs pulses with center wavelength 810 nm ($\approx$ 10 nm bandwidth) at an 80 MHz repetition rate. The laser output is frequency doubled to produce 405 nm light ($\approx$ 3 nm bandwidth) of which 30 mW is focused into a type-0 periodically poled potassium titanyl phosphate (ppKTP) crystal to generate SPDC for E2PA. A small fraction of the pump laser output is routed around the nonlinear crystals and used for C2PA.

We characterize the joint spectrum of the photon pairs with a time-of-flight fiber spectrometer consisting of 500 m-long single-mode fibers and superconducting nanowire single-photon detectors (SNSPDs) (Appendix D). The SPDC is approximately degenerate and centered at 810 nm with $\approx$ 76 nm bandwidth. We determine the entanglement time (Appendix D) in our experiment using the estimated joint temporal intensity, which accounts for the approximately 3700 fs$^2$ of dispersion experienced by each photon pair before reaching the center of the cuvette. The value of $T_e$ at the sample position is $\approx$ 1420 fs. Although $T_e$ is larger than in the ideal (dispersion-free) case, we estimate that lossless dispersion compensation would at most increase the rate of E2PA by a factor of 95 (Appendix D). The SPDC photon rate is measured using a free space coupled single-photon avalanche diode (SPAD). The optical system was designed to minimize losses, thereby minimizing the number of unpaired photons focused into the sample. Taking into account the single-photon detection rate, the photon statistics of the SPDC and the optical losses in our setup from the center of the crystal to the center of the sample ($\approx$ 24%), we estimate that $\approx$ 156 photons per pulse are generated at the output of the crystal and $\approx$ 119 photons per pulse arrive at the sample while operating at our maximum pump power (30 mW) (Appendix D).

Unfortunately, we do not have a direct measurement of entanglement area of our source; we can only estimate that the value of $A_e$ is in the range of 2.1-13.700 $\mu$m$^2$. The estimate of the lower bound is based on the diffraction limit. We find no evidence that the two photons can be focused to a region significantly smaller than that set by the diffraction limit. It has been shown \cite{40, 41} that entangled photons can be focused to a spot size which is a few-fold smaller, however we will neglect these factors here as they have a minor effect in the orders-of-magnitude comparisons we present. Thus, we set the bound using a circular area with radius ($r$) set by the central wavelength of excitation ($r \approx \lambda$). The estimate of the upper bound is set by an elliptical area with diameters set by the measured FWHM of the beam in transverse directions in the center of the sample \cite{42}.

For C2PEF and E2PEF measurements, we use a polarizing beamsplitter (PBS) to combine the SPDC and laser beams, and align them along the same path. The power of the laser beam is controlled using a half waveplate (HWP) in conjunction with the PBS, varying from 0.079-10.5 $\mu$W. The beams are sent through an optical chopper, then focused in the center of a cuvette to a beam FWHM of $\approx$ 68 $\mu$m and $\approx$ 49.0 $\mu$m for the SPDC and laser beams respectively. For E2PEF measurements, we block the laser beam, and for C2PEF measurements we block the SPDC beam. The portion of the beam absorbed in the sample is partially re-emitted as fluorescence, which is collected and focused onto a photon-counting photomultiplier tube (PMT). A combination of a shortpass and bandpass filter (selected for each fluorophore, see Appendix H) in front of the PMT reject scattered 810 nm and 405 nm light. The SPDC beam is found to have a larger divergence within the sample compared to the laser beam (Appendix B). The divergence is taken into account by using the characterization of the geometrical collection efficiency (Appendix H). The geometrical collection efficiency of the fluorescence collection system is characterized using numerical simulations and

\[ A_{ent}^{est} \approx \frac{\lambda^2}{4 \pi} \]
FIG. 2. Schematic of the experimental setup. The 810 nm laser (dark red) is split into two paths; one path is used for C2PEF measurements and the other for E2PEF measurements. The light in the C2PEF path is directed through a half waveplate (HWP) and polarizing beam splitter (PBS) to control the power of light directed to the 2PEF measurement system. The laser is optically chopped (chopper) and focused into a sample. The fluorescence (green) is collected onto a photon-counting PMT and all scattered light rejected using filters (F). The PMT pulses are shaped and sent to a time tagger that is synchronized with the optical chopper. The light in the E2PEF path is frequency doubled (blue) via second-harmonic generation (SHG) and focused into a type-0 ppKTP crystal to generate collinear SPDC photon pairs at 810 nm (light red). Filters (F) are used to remove the remaining 405 nm light. To characterize the joint spectral intensity of the light, a flip mirror directs the pairs into a time-of-flight spectrometer [38, 39]. To characterize the absolute SPDC photon rate at the sample, a HWP and PBS direct the light to a single-photon avalanche diode (SPAD). For E2PEF measurements, the SPDC is sent out of the other output port of the PBS and travels along the same path as the light used in the C2PEF measurements where it is focused into a 2PA sample.

1PEF measurements (Appendix E) and determined to be 15.9% and 4.7% for a point source and line source of fluorescence, respectively. The two beams are found to be misaligned along their path through the sample by ≈ 5 µm horizontally and vertically. As Appendices B and E explain, our experimental apparatus was carefully designed and characterized to be robust against small changes in alignment like these. The longitudinal misalignment between the beams was compensated for.

The six fluorophores investigated in this study are the 1,3,5-triazine-based octupolar molecule “AF455” [43, 44] in toluene, Qdot ITK carboxyl quantum dot 605 (qdot 605) in borate buffer, fluorescein in pH 11 water, the benzothiophene derivative “9R-S” [13] in chloroform, rhodamine 590 (Rh6G) in methanol and coumarin 153 (C153) in toluene (details on sample preparation in Appendix C). These samples are of particular interest because of their well-known and large values of \( \sigma_C \) (see Table I). In addition, two of these samples (Rh6G and 9R-S) were studied in previous reports of E2PA [13, 15].

IV. RESULTS AND DISCUSSION

We measure C2PEF over a range of photon fluxes for all six fluorophores. To use these measurements as a reference for our E2PA detection sensitivity, we compare them with calculations of C2PEF (Appendix H) that use our experimental characterizations and the published C2PA cross-sections (Table I). The measurements strongly agree with the calculations and thus the cross-sections reported in literature. For all six fluorophores we are unable to discern an E2PEF signal. In an analogous manner to the aforementioned calculations, we calculate E2PEF (Appendix H) as a function of the E2PA cross-section to place upper bounds on the E2PA cross-sections of the samples examined here.

Figure 3 shows measured fluorescence count rates as a function of peak photon flux for both laser (blue symbols) and SPDC (red symbols) excitation for all six fluorophores on log-log plots. For all samples, we find the fit (blue line) to the C2PEF signal to have a quadratic power dependence (with an exponent of 2.00 ± 0.05); the signals are thus free of spurious events such as 1PEF or scattered light. For AF455 (Fig. 3(a)), we measure C2PEF down to the lowest peak photon flux of all of the samples, 1.3 × 10^{21} photons cm^{-2} s^{-1}, which is only 590 times larger than our SPDC peak photon flux. The C2PEF of fluorescein, 9R-S, Rh6G and C153 (Fig. 3(c)-(f)) is observed at a minimum flux approximately a factor of 10 higher than for AF455 and qdot 605 (Fig. 3(a),(b)). This minimum flux could be extended to lower values (but not as low as AF455 or qdot 605) if a longer integration time were used for the measurements, which is the case for the AF455 and qdot 605 C2PEF measurements. Fluorescence signals as low as 0.22 cnt s^{-1} should be measurable.
FIG. 3. Measured (blue data points), fitted (blue solid lines) and calculated (black solid and purple dashed lines) fluorescence signal (left vertical axis in cnt s$^{-1}$) for (a) 1.10 mM AF455 in toluene, (b) 8 µM qdot 605 in borate buffer, (c) 1.10 mM fluorescein in pH 11 water, (d) 390 µM 9R-S in chloroform, (e) 1.50 mM Rh6G in methanol and (f) 1.10 mM C153 in toluene. The bottom horizontal axis corresponds to the peak photon flux (photons cm$^{-2}$ s$^{-1}$) of the coherent source (laser) (blue data points) or SPDC source (red data points). On the upper horizontal axis we show the SPDC mean photon number $\bar{N}$ (photons pulse$^{-1}$), which corresponds to the peak photon flux on the lower horizontal axis. A signal below 0.22 cnt s$^{-1}$ is indistinguishable from zero (green region). All E2PEF measurements produce a null result. Solid diagonal black lines show the calculated fluorescence count rate expected for various potential entangled two-photon absorption cross-sections in order-of-magnitude increments (cross-section noted along selected lines) for each fluorophore, assuming that the absorption rate is composed of only the linear photon-flux-dependent term. The purple dashed diagonal line represents the calculated signal using the upper bound on $\sigma_E$ (noted in purple) for each sample.

$^a$ The conversion factor from mean photon number to peak photon flux is different for the coherent source because of its shorter pulse duration and smaller beam size. The laser conversion factor is lower than that for SPDC by a factor of 16.7.
in our experiment (Appendix F). We denote this minimum measurable fluorescence signal as $F_{LB}$. A signal below this level is masked by the noise floor. As mentioned above, we do not observe E2PEF for any of the studied samples. This is demonstrated by the SPDC excitation data points (shown in red) lying below the noise floor (the green region in Fig. 3). For these measurements we use our maximally available pump power to generate an SPDC peak photon flux of $2.2 \times 10^{18}$ photons cm$^{-2}$ s$^{-1}$.

We use our experimental characterizations and the expected linear dependence of E2PA on excitation flux to calculate E2PEF fluorescence signals for various potential values of $\sigma_E$ (Appendix H). The results of these calculations are displayed as black diagonal lines in Fig. 3 with the corresponding $\sigma_E$ value noted along the line. The purple dashed diagonal line corresponds to the fluorescence signal calculated using the cross-section that produces $F_{LB}$ at the peak photon flux of our SPDC source. We denote this cross-section the E2PA cross-section upper bound, $\sigma_{EB}^{UB}$. A summary of $\sigma_{EB}^{UB}$ values is given in Table II and written in purple along the dashed diagonal lines. The sample AF455 has the lowest $\sigma_{EB}^{UB}$ of $1.1 \pm 0.3 \times 10^{-25}$ cm$^2$ while $\sigma_{EB}^{UB} = 350$ GM is among the largest for these samples. The values of $\sigma_{EB}^{UB}$ for fluorescein, Rh6G and C153 differ by less than a factor of two from that for AF455. Many of the parameters for these four samples are similar in magnitude: concentration, quantum yield, fluorescence self-absorption, and the overlap of the emission spectra with the fluorescence collection system’s transmittance spectrum (Appendix H). For 9R-S, the upper bound is one order of magnitude larger, which results from the poor overlap of the emission and system transmittance spectra. For qdot 605, the upper bound is a factor of 11 larger than for 9R-S. This is a result of poor spectral overlap, in addition to a sample concentration two orders of magnitude lower than that used for all other samples. As recommended by the supplier, we use the concentration of qdot 605 as received to avoid compromising the chemical stability of the sample.

The upper bounds our measurements place on $\sigma_E$ range from $10^{-25}$ to $3 \times 10^{-23}$ cm$^2$. These are in stark contrast to the previously reported values of $10^{-21} - 10^{-16}$ cm$^2$ shown in Table I. A particularly illuminating comparison can be made between our result and the published result for samples 9R-S and Rh6G. Using the previously reported $\sigma_E$ values, we estimate the expected E2PEF count rate in our setup. Assuming sample 9R-S has $\sigma_E \approx 2.36 \times 10^{-19}$ cm$^2$ [13], our calculations predict an E2PEF signal of $2.1 \times 10^4$ cnt s$^{-1}$. For Rh6G, a value of $\sigma_E \approx 1.5 \times 10^{-21}$ cm$^2$ [15] predicts an E2PEF signal of $1.8 \times 10^5$ cnt s$^{-1}$ [15]. In either case, we actually measured a signal that is indistinguishable from zero, which is three to five orders of magnitude smaller than expected based on prior reports. One major difference between our experiment and previous experiments is our orders-of-magnitude larger incident SPDC photon rate ($\approx 10^{10}$ photons s$^{-1}$). Although $A_e$ and $T_e$ likely vary between experiments, we have no reason to believe these will differ by the many orders of magnitude required to explain this discrepancy.

We compare our C2PEF and E2PEF results to determine an upper bound on the “quantum advantage” of 2PA (QA$^{UB}$). As previously mentioned, we define the quantum advantage as the ratio of the minimum photon flux required to observe C2PA to that for E2PA. By extrapolating our C2PEF fit to $F^{LB}$ for the sample AF455, for example, we determine that C2PEF should be measurable down to $8.5 \times 10^{20}$ photons cm$^{-2}$ s$^{-1}$. E2PEF is not measurable at our maximum SPDC photon flux, $2.2 \times 10^{18}$ photons cm$^{-2}$ s$^{-1}$, but might be measurable at a higher photon flux. Thus, QA$^{UB}$ of 2PA for this sample is 380. Values of QA$^{UB}$ for all of the samples (Table II) range from 380 – 7000, in contrast with previous reports of QA values as large as $10^{10}$ [10]. It is worth mentioning that although the QA can be increased if $A_e$ and $T_e$ are decreased while the properties of the laser beam remain fixed, a many-orders-of-magnitude increase is unlikely. We also point out that large oscillations in $\sigma_E$ as a function of $T_e$ (“entangled two-photon transparencies” [6]) have been theoretically predicted for some molecular fluorophores [37], but not for the molecules considered here, and furthermore it seems very unlikely that we are at orders-of-magnitude deep minima for all six fluorophores.

Table II also shows estimates of the E2PA cross-section for the six fluorophores, $\sigma_{EB}^{BT}$. These estimates are based on the relation given in Eq. (7) using the $\sigma_E^{10 \text{nm}}$ given in Table II and our estimates of $T_e$ and $A_e$ specified in Section III. We use the lower bound of $A_e$ in this estimation to show the largest value $\sigma_{EB}^{BT}$ could take on. These values are three to five orders of magnitude below our established cross-section upper bounds. These estimates provide a reference for the cross-section sensitivity necessary to observe E2PA.

There are other publications in this field that support our findings. In particular, Ashkenazy et al. [10], argued that using “typical” values of $A_e$ (50 $\mu$m$^2$) and $T_e$ (50 fs), they can estimate $\sigma_E \approx 10^{-29}$ cm$^2$ for metallic nanoparticles with a large $\sigma_C \approx 100$ GM. Cross-sections of this size are in agreement with our established bounds of $\sigma_E$. Another interesting example is provided in the recent work by Li et al. [47] who used a single setup to measure both C2PEF and squeezed-light 2PEF (SL2PEF) of the samples DCM in dimethyl sulfoxide and fluorescein in pH 13 water. The squeezed light generated by four-wave mixing in a Rubidium vapor cell could be adjusted over a range of excitation powers (0.03-8 mW). The SL2PEF signals for DCM and fluorescein were factors of $\approx 2.0 - 2.8$ and $\approx 47$ larger, respectively, than the C2PEF signals at the same excitation flux. The authors did not report values for cross-sections. However, these significant but modest enhancements and the fact that measurements were performed with a squeezed light source that provides four to six orders of magnitude higher excitation flux than an SPDC source, is consistent with the upper bounds established in our study.
V. CONCLUSIONS

In this report, we discussed important aspects of designing and implementing a fluorescence-based E2PA measurement. We presented an experimental apparatus for measuring E2PEF and C2PEF in nearly identical experimental conditions. The results from C2PEF serve as a vital reference point for the capability of our fluorescence system. Using C2PEF, 1PEF, numerical simulations and calculations, we determined the sensitivity of our E2PEF measurements. Although we do not observe an E2PEF signal, our results set upper bounds on $\sigma_E$ of the six chosen fluorophores in the range of $10^{-25} - 3 \times 10^{-23} \text{ cm}^2$. Two of these samples have published $\sigma_E$ values that are nearly four and five orders of magnitude larger than the upper bounds we report.

We emphasize that $\sigma_E$ depends on spatio-temporal properties of the excitation source, unlike $\sigma_C$. Without knowing the entanglement area and entanglement time, the cross-sections measured in different experimental apparatuses cannot be compared with one another. For our source, we estimated a range within which our entanglement area is constrained, 2.1-13.700 $\mu$m$^2$, and we estimated the entanglement time, 1420 fs based on our measured SPDC spectrum and estimated group delay dispersion. While we had hoped to measure these quantities directly, in lieu of this we made explicit the details of our setup and the assumptions that went into the estimation of these quantities.

Our results differ significantly from previous E2PA publications using SPDC excitation. Our evidence indicates that E2PA cross-sections are orders of magnitude smaller than previously claimed \cite{7-11}. As we demonstrated in this report, the clarification of the inconsistencies in the field is underway. This is an important step forward in the quantification of the achievable “quantum advantage” and thus the merit of E2PA for spectroscopy and imaging applications.

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K.M.P and A.M. contributed equally to this work.

Appendix A: Detailed experimental setup and parts

In Fig. 4 we show a detailed diagram of our setup with labeled parts. We list the part numbers below.

Main source

- Laser source = Chameleon Discovery Laser
- SHG = VUE Harmonics SHG unit

Pair source

- HWP1 = Zero-order half waveplate 405 nm (Thorlabs WPH05M-405)
- Pol = Glan laser calcite polarizer (Thorlabs GL10-A)
- F1 = three dichroic mirrors (2 x 10Q20BB.1 and TLM-400-458-1025), three interference bandpass filters (3 x Thorlabs FBH405-10) and one colored glass filter (Thorlabs FGB37M)

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TABLE II. Summary of literature C2PA cross-sections at 810 nm ($\sigma_{C}^{810\text{ nm}}$) and our established E2PA cross-section upper bounds ($\sigma_{C}^{UB}$), quantum advantage upper bounds ($\sigma_{\text{QA}}^{UB}$), and estimates for the E2PA cross-section ($\sigma_{E}^{est}$) (based on Eq. 7) using $T_e = 1420$ fs and $A_e = 2.1 \mu$m$^2$.

| Sample | $\sigma_{C}^{810\text{ nm}}$ (GM) [Ref.] | $\sigma_{E}^{UB}$ ($10^{-25} \text{ cm}^2$) | $\sigma_{\text{QA}}^{UB}$ | $\sigma_{E}^{est}$ ($10^{-29} \text{ cm}^2$) |
|--------|------------------------------------------|------------------------------------------|------------------------|------------------------------------------|
| AF455  | 350 ± 30 [18]                           | 1.1 ± 0.3                                | 380 ± 130              | 12                                       |
| Qdot 605 | 27000 ± 8000 [18]                       | 270 ± 60                                 | 700 ± 200              | 910                                      |
| Fluorescein | 21 ± 2 [18]                           | 1.5 ± 0.4                                | 1900 ± 600             | 0.70                                      |
| 9R-S   | 27.9 ± 1 [18]                           | 24 ± 6                                   | 7000 ± 2000            | 0.94                                      |
| Rh6G   | 78 ± 7 [18]                            | 1.8 ± 0.4                                | 1100 ± 400             | 2.6                                       |
| C153   | 17 ± 2 [18]                            | 1.9 ± 0.5                                | 2300 ± 800             | 0.57                                      |

$^a$ Measured at 800 nm
FIG. 4. Detailed diagram of our experimental setup. See main text for abbreviation definitions and part numbers.

- L1 = 300 mm focal length lens (Thorlabs LA4579-A)
- ppKTP crystal (Raicol Crystals Ltd., type-0 SHG AR coated 3.425 μm poling period, 10 mm long)
- crystal temperature controller (Covesion PV10) set to 30.00°C ± 0.01°C
- F2 = interference longpass filters (Semrock BLP01-442R-25, BLP01-633R-25 and 3 x FF01-496/LP)
- L2 = 200 mm focal length lens (Thorlabs LA1979-B)
- F3 = dichroic mirrors (2 x ARO MR6040) and interference longpass filter (Thorlabs FELH0700)
- L3 = 12.7 mm focal length achromatic doublet (Thorlabs AC064-013-B)
- fiber beamsplitter (Thorlabs FC830-5OB-FC)
- SMF = 2 x 500 m-long single mode fiber (Nufern 780-OCT)
- SNSPDs = Superconducting nanowire single-photon detectors (Quantum Opus, LLC, Opus One, optimized for the 850-1200 nm wavelength region) with a detection efficiency of ≈ 75% at 810 nm
- closed-cycle helium cryocooler (Sumitomo HC-4E2)
- temperature monitor (SIM 922)
- detector bias and readout modules (Quantum Opus, LLC, QO-SIM-CRYO)
- time tagger = picosecond event timer and time-correlated single photon counting system (PicoQuant HydraHarp 400)

Photon rate measurement (details on use in Appendix D)
- L4 = 50.2 mm effective focal length lens (Newport KPX082AR.16)
- SPAD = single-photon avalanche diode (PerkinElmer SPCM-AQR-14)
- counter = Timer/Counter/Anaylzer (Tektronix FCA3103)

2PEF measurements (details on use in Appendix F)
- optical chopper head and controller (New Focus 3501 Optical Chopper)
- L7 = 50 mm focal length lens (Thorlabs LA1131-B)
- UV quarts sample cuvette with 2 mm width × 10 mm path length (FireFlySci, 1FLUV2)
- machined cuvette holder designed for stability and low footprint to bring optics close to excitation volume
- L9, L10, L11 = Collection Optic with High Numerical Aperture (COHNA) lens system [48]
- F4 = Shortpass filter (Semrock FF01-758/SP-25 (pass below 758 nm)) and sample-dependent bandpass filter (AF455 and C153 - Semrock FF02-470/100-25 (100 nm bandwidth centered at 470 nm), qdot 605 and 9R-S - Chroma ET610/75m (75 nm bandwidth centered at 610 nm), fluorescein and Rh6G - Semrock FF01-535/150-25 (150 nm bandwidth centered at 535 nm)) (filter spectra is shown in Fig. 8)
- SM = spherical mirror with 15 mm effective focal length, 35 mm diameter (Edmund Optics, #43-467)
- PMT = photon-counting metal package photomultiplier tube (Hamamatsu H10682-210)
- thermoelectric cooler (CP40336) to cool PMT to 5°C
- time tagger = picosecond event timer and time-correlated single photon counting system (PicoQuant HydraHarp 400)

Beam alignment and characterization (details on use in Appendix B)
- L7 = 50 mm focal length lens (Thorlabs LA1131-B)
- L12 = 50 mm focal length lens (Thorlabs LA1131-B)
- L13 = 62.9 mm effective focal length lens (Newport KPX085AR.16)
- cam1 = UI-3590LE-C-HQ
- cam2 = Thorlabs UI-224XSE

1PA source (details on use in Appendix E)
- 458 nm source = OBIS 458 LX
- F6 = neutral density (ND) filter wheel (Thorlabs)

Other parts
- HWP2 = Zero-order half waveplate 808 nm (Thorlabs WPH10M-808)
- HWP3 = half waveplate 800 nm (Tower Optical)
- L5 = 88.3 mm effective focal length lens (Newport KPX091AR.16)
- L6 = 75 mm effective focal length lens (Newport KPC037AR.16)
- PBS = polarizing beam splitting cube (Thorlabs PBS122)
- F4 = longpass interference filter (FELH0700)

Appendix B: Alignment details

A telescope (L5 and L6) is used to resize the laser beam to the SPDC beam size. The alignment of the beam into the sample is checked using two cameras (cam1 and cam2). Lens L7 and L8 are placed approximately the same distance from the flip mirror, enabling a view on cam1 of the beams at and near the focus in the cuvette. With this camera, we check the alignment of the beams through alignment irises, and measure beam size, Rayleigh range and overlap of the laser and SPDC beams. Two lenses after the sample (L12 and L13) collimate and focus the beam onto cam2. With this camera we verify that the beams remain overlapped and centered along the x- and y-directions after passage through the cuvette.

A typical transverse spatial overlap of the two beams at the focus (cam1) in the sample is shown in Fig. 7. The centers of the laser and SPDC beam are misaligned from one another by ≈ 5 µm vertically and horizontally. Zemax simulations (Fig. 7) indicate that misalignments of this magnitude have no effect on the collection efficiency. The beams’ centers on cam2 are also overlapped within ≈ 5 µm vertically and horizontally. The foci of the beams is roughly centered along the z-direction by placing the lens L8 one focal length away from the center of the cuvette. On cam1 we observe a shift between the focuses of the laser and SPDC beam in the z-direction of ≈ 500 µm. We compensate for this by shifting lens L8 so that either beam’s focus along the z-direction is in the center of the cuvette prior to measurements. The beam overlap is checked regularly.

The COHNA lens system (L9, L10 and L11) and filters (F5) are contained within a 25.4 mm-diameter lens tube. The spacing of the optics in the lens tube is based on Ref. [48]. The COHNA lens system (and filters) and the spherical mirror (SM) are each placed on a three-axis stage and roughly aligned in the three directions based on Zemax spacings. To check that the alignment of the system is optimized, we first adjust lens L8 to optimize C2PEF and thus the geometrical collection efficiency of the system. Next, the COHNA lens system and the spherical mirror are each adjusted to maximize C2PEF. This process is iterated until the collection efficiency is optimal. A CW 458 nm source excites 1PA in the sample to aid in the characterization of the geometrical collection efficiency (Appendix E).

A SwampOptics Grenouille 8-50-USB is employed to measure the FWHM pulse duration (τ) of the laser beam.

Appendix C: Sample preparation details

The “AF455” fluorophore [43, 44] was provided by Drs. T. Loon-Seng Tan and T. Cooper from the Air Force Research Laboratory. Fluorescein, rhodamine 590 (6G) and coumarin 153 (540A) were ordered from Sigma-Aldrich and used as received. Qdot ITK Carboxyl Quantum dot 605 (Qdot 605) in borate buffer was ordered from ThermoFisher, stored at 4°C and only used for six months after receiving. The thienoacene fluorophore “9R-S” [13] was provided by Prof. Theodore Goodson from the University of Michigan. Various solvents were used to prepare the samples including toluene (≥ 99.98%), pH 11 water (Hydrion pH 11 buffer capsule in distilled water), methanol (≥ 99.9%), ethanol (≥ 99.5%), and chloroform (≥ 99.9%). The concentration and absorption/emission spectra were checked using a UV-VIS-NIR spectrophotometer (Agilent Cary 5000 Scan) and a fluorometer (Horiba Fluorolog-3 FL3-222). The absorption and emission spectra were compared with published spectra to ensure the sample is not contaminated, degraded, etc.
FIG. 5. (a) Measured joint spectral intensity (JSI) where $\lambda_{S,I}$ are the signal and idler wavelengths. (b) The JSI is projected onto the horizontal axis and vertical axis showing the signal (blue) and idler (red) spectra respectively. The FWHM of the signal and idler spectra are 79 and 72 nm respectively. The overlap of the spectra is evident in the dark red region. (c) Estimated joint temporal intensity (JTI) obtained through a discrete Fourier transform as described in the main text. (d) Projection onto the antidiagonal axis, $t_S-t_I$, of the JTI shown in (c) (blue) and for a transform limited ($\beta = 0 \text{ fs}^2$) JTI (red). The FWHM of these projections are 1420 fs and 20 fs.

**Appendix D: SPDC source characterization**

The joint spectral intensity (JSI) distribution for our SPDC source is measured using a fiber-based time-of-flight spectrometer. We follow the procedure detailed in Refs. [38, 39]. We measured the dispersion of the fiber over the wavelength range from 680 to 1200 nm. At 810 nm, the center wavelength of the SPDC source, the fiber’s dispersion is $-0.114 \text{ nm nm}^{-1} \text{ km}^{-1}$. Using this and the known timing information, we obtained the normalized JSI shown in Fig. 5(a). The shape indicates the expected wavelength anticorrelation of SPDC. Taking the projection of the JSI on the vertical and horizontal axis (signal and idler projections, or vice versa for degenerate type-0 SPDC) revealed several results, see Fig. 5(b). The vertical and horizontal projections are shown in red and blue, respectively, with FWHM of 72 and 79 nm. The various features in the spectra and the detuning from degeneracy is likely a result of a combination of measurement artifacts, such as the spectral profiles of the optics.

The entanglement time $T_e$ was estimated as the FWHM of the antidiagonal projection of the joint temporal intensity (JTI) [4]. We did not measure the JTI directly; instead we calculated it based on our measured JSI. Computing the JTI from the JSI requires knowledge of the spectral phase of the SPDC. We did not have a measurement of this phase; instead we estimated the accumulated group delay dispersion ($\beta$) of the pulse from the center of the crystal to the center of the sample to be 3700 fs$^2$. We set the joint spectral amplitude (JSA) in the frequency domain to the square root of the JSI in the frequency domain multiplied by the phase factor due to $\beta$, $\text{JSA} = \sqrt{\text{JSI}} e^{i\beta(\omega_{S} - \omega_{P}/2)^2/2} e^{i\beta(\omega_{I} - \omega_{P}/2)^2/2}$ where $\omega_{S}$, $\omega_{I}$, and $\omega_{P}$ are the frequencies of the signal, idler, and pump fields, respectively. In asserting this, we assumed that the SPDC was transform limited in the center of the crystal and that the only significant accumulated phase factor was that due to $\beta$. We Fourier transformed the JSA to obtain the joint temporal amplitude (JTA). The magnitude squared of the JTA gave us the joint temporal intensity (JTI) shown in Fig. 5(c). The projection of the JTI onto the antidiagonal ($t_S - t_I$) is shown in blue in Fig. 5(d).

We find that $T_e \approx 1420$ fs. This can be compared with a transform-limited ($\beta = 0 \text{ fs}^2$) pulse (Fig. 5(d) in red) that has $T_e \approx 20$ fs. The projection of the JTI onto horizontal and vertical axes both have FWHM’s of 1040 fs. Since this width is significantly larger than the pump pulse du-
ratiort, it is a good approximation for the pulse duration of signal and idler beams.

To calculate the advantage of dispersion compensation or a dispersion-free setup on the rate of E2PA in our setup, we consider how dispersion affects the SPDC’s fulfillment of the simultaneity requirement of 2PA. This simultaneity requirement asserts that two photons must arrive at the fluorophore within a time window ($\Delta t$) set by the fluorophore’s virtual state lifetime. For a dispersed pulse, fewer of the SPDC photon pairs will arrive within this time window. A precise calculation of the virtual state lifetime of these large molecular fluorophores is not feasible, however we can consider any arbitrary time window. In Fig. 6(a) a zoomed in version of the projection of the JTI from Fig. 5(d) is shown with a time window, $\Delta t = 50$ fs, indicated in yellow. The number of photon pairs of the transform limited SPDC ($\beta = 0$ fs) which satisfy $|t_S - t_I| \leq \Delta t$ divided by the number of photon pairs of the dispersed SPDC ($\beta = 3700$ fs$^2$) which satisfy that same constraint (coincidence ratio) is shown in Fig. 6(b) as a function of $\Delta t$. A yellow bar indicates the 50 fs time window shown in Fig. 6(a). For the smallest possible $\Delta t$ we can consider based on our resolution (1 fs), the coincidence ratio is 95. Thus a factor of 95 more photon pairs of the transform limited SPDC satisfy $|t_S - t_I| \leq 1$ fs than for the dispersed SPDC. This implies that for the shortest virtual state lifetime we can consider (1 fs), dispersion compensation or a dispersion-free setup would at most improve the E2PA rate by a factor of 95. If the virtual state lifetime is longer, the factor is smaller as indicated in Fig. 6(b).

It is worth noting that we could have reduced the $T_e$ of our source by reducing our SPDC bandwidth using a bandpass filter which is narrower than the $\approx 76$ nm width. This would consequently reduce our photon flux. We did not try this as it seems unlikely that a decreased photon flux would increase our likelihood of measuring E2PA. Although some of our photons are likely dispersed too far in time to be useful for E2PA, they will not decrease our chances of measuring E2PA with the fraction of photons still satisfying the simultaneity requirement.

Appendix E: Fluorescence collection efficiency

Initial characterization of the fluorescence system’s geometrical collection efficiency is performed using Zemax’s OpticStudio. The solvent, glass cuvette walls, four collection optics and detector surface are modelled in the program. Using a merit function and an optimization algorithm, we find the ideal spacing of the optics. Since a 2PA process can only occur if two photons are sufficiently well-spatially overlapped at a fluorophore, the excitation rate of C2PA and E2PA depend on the focusing of the respective beams. Propagating a beam that is small in area into a 2PA sample will more frequently lead to the overlap of two photons at a fluorophore than using a beam with the same photon rate that is larger in area. For C2PA, this is clearly evident through the quadratic photon flux dependence in the excitation rate (Eq. (3)), where the photon flux depends inversely on the beam size. For E2PA, this spatial dependence is hidden, since the excitation rate depends linearly on photon flux (Eq. (6)) in a similar manner to 1PA (a beam size independent process). The spatial dependence is instead included in the E2PA cross-section (Eq. (7)), which depends inversely on the entanglement area. In our experiment, the excitation beams are not collimated (see divergence of the SPDC (green) and laser (blue) beams in Fig. 7(a)) and thus the excitation volume is a non-trivial shape. In order to properly calculate the rate of collection of C2PEF and E2PEF (Appendix H), it is critical to characterize the collection efficiency of our system as a function of the origin of the fluorescence within the cuvette along the $z$-direction. Ideally we would also take
into account the collection efficiency as a function of the origin of the fluorescence in the $x$- and $y$-directions, however as we discuss below the transverse dependence is less critical to the final result.

In Zemax we simulate the collection efficiency as a function of the origin of the fluorescence within the cuvette volume. We model a point source of fluorescence that emits rays isotropically at some position in the cuvette. The number of those rays collected onto the detector are counted. We systematically translate this source in all directions to trace out contour plots of collection efficiency in the $x$ and $z$ plane (centered in $y$) (Fig. 7(a)) and $x$ and $y$ plane (centered in $z$) (Fig. 7(b)). In this figure we rescale the collection efficiency found through Zemax to match experimental values, as discussed below.

We find that the collection efficiency is slightly asymmetric in the $x$ direction, collecting slightly better when the point source is displaced towards the PMT. Experimentally, we ignore this minor asymmetry and center the beams through the cuvette.

Although the spatial distributions of the excitation beams have some transverse extent, we note that in Fig. 7 it is clear that transverse displacements from $x = y = 0$ must be large (> 10$^2$ mm at $z = 0$) and transverse displacements nearly negligible beyond $|z| > 1$ mm) to significantly affect the collection efficiency. Our excitation beams’ spatial distributions in the transverse directions are contained within a region of nearly constant collection efficiency, thus in our calculations (Appendix II), we ignore the transverse spatial distribution of the excited fluorescence.

In Zemax, we simulate the total collection efficiency ($\kappa$) of a particular excitation volume for the limiting cases of a uniform cylindrical excitation volume (50 µm diameter) that extends the length of the cuvette (centered in $x$ and $y$) and for that of a point source centered in the cuvette. For the former, the collection efficiency is at a minimum for the system, $\kappa_{\text{min}} = 6.1\%$, and for the later, the collection efficiency is at a minimum for the system, $\kappa_{\text{max}} = 20.9\%$.

To a good approximation, the collection efficiency, $K$ (found using Zemax) as a function of $z$ (cm), fits to a complementary error function. This can be qualitatively understood by the similarity of the simulation of the collection efficiency as a function of $z$ to a knife’s edge beam profile measurement, which fits the same type of function. In both cases we measure intensity as a function of the placement of an object. This object alters the intensity passed to a detector. Thus, the collection efficiency as a function of $z$ takes the form

$$K(z) = \frac{\kappa_{\text{max}}}{2} \text{erfc} (\alpha (|z| - z_0)),$$

where $\kappa_{\text{max}} = 0.20$, $\alpha = 2.8$ cm$^{-1}$ and $z_0 = 1.5$ cm. These parameters are set by Zemax collection efficiency simulations for the translation of a point source along the $z$ direction (centered in $x$ (cm) and $y$ (cm)), and $z = 0$ is the center of the cuvette. The function $K(z)$ is used to characterize the portions of an excitation volume extended along the $z$ direction that contribute to the collected fluorescence signal. Below we discuss our method to adjust $\kappa_{\text{max}}$ to fit our experiment.

We measure the minimum collection efficiency, $\kappa'_{\text{min}}$ (where $'$ indicates the experimental value rather than simulated), of our fluorescence setup using a 1PEF-based technique. In a similar manner to the treatment in Ref. [36], the measured one-photon fluorescence rate, $F_{\text{1PEF}}$ (cm$^{-1}$), can be described by

$$F_{\text{1PEF}} = (1 - 10^{-OD}) \frac{W}{h\nu} \kappa'_{\text{min}} \int_{\lambda_i}^{\lambda_f} \gamma(\lambda) \Phi(\lambda) d\lambda,$$

where $OD = \epsilon cl$ is the optical density of the sample at the excitation wavelength, $\epsilon$ (cm$^{-1}$ M$^{-1}$) is the extinction coefficient of the sample at the central excitation wavelength, $c$ (M) is the concentration of the sample, $l$ (cm) is the cuvette length [39], $W$ (W) is the average power incident on the sample, $h\nu$ (J) is the average energy of an incident photon, $\lambda_i, \lambda_f$ (nm) are initial and final wavelengths chosen to integrate over the entire emission spectrum of the sample, $\gamma(\lambda)$ is the wavelength-dependent component transmission efficiency (detector, filters, lenses and cuvette), and $\Phi(\lambda)$ (nm$^{-1}$) is the differential quantum yield. The minimum collection efficiency, $\kappa'_{\text{min}}$, characterizes a system with a nearly uniform cylindrical excitation volume extending the length of the cuvette, thus we use an excitation source which satisfies this requirement. A bandpass filter (F5) in front of the PMT rejects scattered light. The concentration of the sample is chosen to be relatively low ($\approx 0.1 - 10\mu$M) to reduce the need to characterize fluorescence self-absorption in the sample.

We estimate $\gamma(\lambda)$ as

$$\gamma(\lambda) = \prod_{i=1}^{N} T_{\text{filter}}(\lambda) \prod_{j=1}^{M=3} T_{\text{lens}}(\lambda) T_{\text{cuvette}}(\lambda) \times \frac{1}{2} \left(1 + T_{\text{cuvette}}^2(\lambda) R_{\text{phkM}}(\lambda)\right),$$

where $T$ and $R$ are the transmittance and reflectance specified from the manufacturer of a given optic. In the 1PEF measurement we use one filter and thus $N = 1$. A normalization of quantum yield is used such that $\int_0^\infty \Phi(\lambda) d\lambda$ gives the value published in literature for the total quantum yield of the fluorophore (Table III). For these 1PEF measurements, the laser excitation, fluorophore absorption and emission, PMT quantum efficiency and filter spectra are shown in Fig. 8(g)-(h).

A CW 458 nm source is employed for this 1PEF measurement. The amount of power reaching the sample is measured after L8 and varied using the ND wheel (F6) after the output of the laser. The samples chosen for this measurement are Rh6G in ethanol and fluorescein in pH 11 water. First, the laser is sent through just the solvent measurement are Rh6G in ethanol and fluorescein in pH 11 water. First, the laser is sent through just the solvent.
FIG. 7. Illustration of the geometrical collection efficiency inside of the cuvette and laser/SPDC beam overlap. (a) A cross-section of the cuvette in the $xz$ plane. The selected contours show where the collection efficiency is constant, based on Zemax simulations. The magnitude of the collection efficiency is scaled based on 1PEF measurements. The beam propagation is shown for the laser beam (blue) and the SPDC beam (green). (b) A cross-section of the cuvette in the $xy$ plane. The expanded inset is a view of the center of the $xy$ plane showing approximate beam FWHMs and overlap.

Next, the laser is sent through the sample and a signal is measured. The fluorescence signal is measured at six different excitation powers ranging from 10 - 150 nW. The beam size and Rayleigh range at the focus on cam1 is measured to be 15 $\mu$m and 1 mm respectively. This Rayleigh range suggests that the beam size will be significantly larger at the edge of the 10 mm path length cuvette compared to at the center. However, the beam has a small transverse spatial extent for all $z$ (at $|z| = l/2$ the beam size is about $\approx 4\%$ the cuvette width) relative to the collective efficiency contour spacing in the transverse direction. Thus, the excitation volume can be approximated as a uniform cylindrical volume that extends the length of the cuvette. Using the comparison of $\kappa_{\text{min}}$ to $\kappa_{\text{min}}$ we rescale the maximum collection efficiency of the system to account for the experimental imperfections, $\kappa'_{\text{max}} = \kappa_{\text{min}}/\kappa_{\text{min}} \times \kappa_{\text{max}}$, which modifies Eq. (E2). We measured an average $\kappa'_{\text{min}} = 3.9 \pm 0.6\%$ and $5.4 \pm 0.7\%$ for Rh6G and fluorescein respectively. Using the average of these two, we find $\kappa'_{\text{max}} = 15.9\%$.

Appendix F: Data acquisition, error bars and sensitivity bound

In this section we describe the details of data acquisition for C2PEF and E2PEF measurements. First we describe our fluorescence background subtraction method. Next we describe how C2PEF measurements were performed. Afterwards we describe the choice of integration times for E2PEF measurements and how those measurements were performed. Lastly we describe how the measured quantities, error bars and sensitivity bound on Fig. 3 were determined.

The laser and SPDC beams are optically chopped to perform on-the-fly background subtraction on the fluorescence signal. The timetagger histogram is used to subtract the background (chopper blade blocking beam) from the signal (chopper blade passing beam). We calibrate this background subtraction method using a strong C2PEF signal. For $\approx 5\%$ of the measurement runtime the chopper blade is neither completely blocking nor passing the beam; this portion of the measurement is discarded.

For C2PEF measurements, the laser power is controlled using a motorized half-waveplate (HWP3). The power is measured (Thorlabs S130C power sensor and PM100D meter) by flipping the sensor into the beam using a motorized flip mount that ensures repeatable positioning. The power sensor and meter are compared with a calibrated photodiode to determine the correction factor necessary for absolute power readings. At each power, 3-5 C2PEF measurements are performed. The integration times at higher powers are 30 seconds and at lower powers are 30 minutes. The long integration time is necessary to measure signals as low as 0.22 cnt s$^{-1}$.

We characterize the stability of the fluorescence measurements using an Allan deviation analysis, and base our measurement integration time for the E2PEF measurements on the result. To do this, we place the 1.10 mM fluorescein sample in the cuvette, unshutter the laser beam and measure the C2PEF signal every minute for one 14 hour period overnight and one 11 hour period during the
day. We use this data to check the Allan deviation at various integration times. The Allan deviation is found to have a minimum at 45 minutes integration time.

For E2PEF measurements, the SPDC pump laser power is set to 30 mW and is monitored periodically. Three E2PEF measurements are performed on each sample. These measurements are each 45 minutes long. We also block the beam periodically and take a 45 minute background measurement. We compare this measurement with those with the beam unblocked to look for significant changes in the signal. We found no changes.

The C2PEF measurements are averaged for each sample at each power. The E2PEF measurements are averaged for each sample. These averages are displayed on Fig. 3. The corresponding vertical error bars are assigned in a systematic way. First, we compare the standard deviation of the set of measurements to the sets’ uncertainty due to Poisson counting statistics. The larger of these two values is multiplied by two (coverage factor $k = 2$) and used for the vertical error bar.

The horizontal error bars correspond to the uncertainty in peak photon flux (bottom axis), which is larger than the uncertainty in photon rate (top axis). This larger uncertainty arises because of the additional uncertainty in the beam size and pulse duration. The uncertainty in the photon rate, beam size and pulse duration is propagated to give an uncertainty in peak photon flux. A coverage factor $k = 2$ is again used to achieve $\approx 95\%$ confidence that the true value lies within the bounds set by the error bars.

The lower bound of our measurable fluorescence rate, $F_{\text{LB}}$, is quantified by first determining the minimum C2PEF signal that is distinguishable from zero. We measure C2PEF at rates as low as $0.38 \pm 0.24 \text{ cnt s}^{-1}$ that agree well with the quadratic fit of the data measured at higher excitation flux. This sets our confidence in signals at least as low as $0.38 \text{ cnt s}^{-1}$. Next, we measure “zero signal”, to determine what should we expect in the absence of signal. To do this, we place the 1.10 mM fluorescein sample in the cuvette, unshutter the SPDC beam and subsequently block the SPDC beam using black aluminum foil tape (Thorlabs T205-1.0) placed after filters F3. We then acquire data for 405 minutes, or nine 45 minute measurements. The purpose of blocking the beam instead of shuttering it is to serve as an additional check for scattered light entering the detector. The fluorescein sample aided in this purpose by serving as a source that could be excited by the scattered light.

It was clear from these measurements that no stray signals enter the detector. From this, our $F_{\text{LB}}$ is set to $0.22 \text{ cnt s}^{-1}$ ($2\sigma$ from zero) with $\approx 95\%$ confidence. $F_{\text{LB}}$ sets the vertical position of the light green region in Fig. 3.

#### Appendix G: Effects of loss

In the case of linear loss between the SPDC generation crystal and the sample, the 2PA rate (Eq. 1) is modified [50]

$$ R = k_2 T^2 \left\langle \hat{a}^\dagger \hat{a}^2 \right\rangle = k_2 T^2 \mu^2 g^{(2)}, \quad (G1) $$

where the linear loss has been modeled as a lossless beam-splitter with transmittance $T$. For excitation with a single-mode squeezed vacuum,

$$ R = \frac{1}{2} \sigma C T^2 \left( \frac{\phi_{\text{sample}}}{\tau A} + 3\phi_{\text{sample}}^2 \right). \quad (G2) $$

where $\phi_{\text{sample}}$ is the photon flux in the SPDC crystal (note: photon flux, not photon pair flux). Rewriting in terms of the photon flux at the sample ($\langle \phi_{\text{sample}} = T \phi_{\text{sample}} \rangle$) yields

$$ R_E = \frac{1}{2} (\sigma E T \phi_{\text{sample}} + 3\sigma C \phi_{\text{sample}}^2). \quad (G3) $$

To extract $\sigma_E$, the flux at the sample should be scaled by $T$. This loss scaling signature of E2PA was also noted in works by Dayan et al. [51, 52] and can be used as a method to confirm E2PA.

#### Appendix H: Calculating upper bounds of the E2PA cross-section

Here we describe the equations relevant for the calculation of the E2PA cross-section upper limit. First we present the description of the C2PEF signal using sample parameters and experimental characterizations. Next we compare the results of the C2PEF calculation based on this description to the measured signal. We use the comparison for determination of fluorescence self-absorption in the sample, an efficiency that was not experimentally determined. Finally we describe the E2PEF signal, and how it can be used for estimation of the E2PA cross-section upper bound.

The C2PEF signal, $F_C$ (cnt s$^{-1}$), measured in our experiment can be described by

$$ F_C = g f(c) \int_{-l/2}^{l/2} N_C(z) K(z) d\tau \int_{\lambda_i}^{\lambda_f} \gamma(\lambda) \Phi(\lambda) d\lambda, \quad (H1) $$

where $g$ (MHz) is the pulse repetition rate, $f(c)$ is the concentration ($c$ ($\mu$M)) dependent fraction of fluorescence which is not reabsorbed in the sample, $N_C(z)$ (fluorophores cm$^{-1}$ pulse$^{-1}$) is the number of fluorophores excited per infinitesimal length $dz$ (cm) per laser pulse, $l$ (cm) is the cuvette path length, $K(z)$ is the geometrical collection efficiency as a function of $z$ (cm) as described in Eq. (E3), $\gamma(\lambda)$ is the component transmission efficiency as described in Appendix E (where here $N = 2$).
and \( \Phi(\lambda) \) (nm\(^{-1}\)) is the differential fluorescence quantum yield. A proper normalization of quantum yield is used such that \( \int_{0}^{\infty} \Phi(\lambda)d\lambda \) gives the value published in literature (Table 11) for the total quantum yield of the fluorophore. The integration limits for the \( \lambda \) (nm) integral are set so that the integral spans over the entire emission spectrum of the fluorophore. The laser excitation, fluorophore emission, PMT quantum efficiency and filter spectra are shown in Fig 8 a)-(f). We can further define \( N_C(z) \) as

\[
N_C(z) = \frac{1}{2} \sigma_C n \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \phi(x, y, z, t)^2 dx dy dt, \tag{H2}
\]

where \( \sigma_C \) (cm\(^4\) s photon\(^{-1}\)) is the C2PA cross-section, \( n \) (fluorophores cm\(^{-3}\)) is the number density of fluorophores and \( \phi(x, y, z, t) \) (photons cm\(^{-2}\) s\(^{-1}\)) is the photon flux of the laser beam. The factor of 1/2 accounts for the requirement of two photons absorbed per one fluorophore excited. Equation (H2), is related to the familiar phenomenological C2PA excitation rate, \( R \) (s\(^{-1}\) fluorophore\(^{-1}\)), described in Eq. (3) by

\[
N_C(z) = n \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} R dx dy dt, \tag{H3}
\]

with \( R \) having implied dependence on \( x \) (cm), \( y \) (cm), \( z \) and \( t \) (fs).

The temporal and transverse spatial profiles of the laser beam or SPDC beam can be approximated by Gaussians, giving \( \phi(x, y, z, t) \) the form

\[
\phi(x, y, z, t) = \phi_0(z) \text{Exp} \left( -4\ln2 \frac{t^2}{\tau^2} \right) \times \text{Exp} \left( -4\ln2 \frac{x^2}{\Delta x(z)^2} \right) \times \text{Exp} \left( -4\ln2 \frac{y^2}{\Delta y(z)^2} \right), \tag{H4}
\]

where \( \phi_0(z) \) (photons cm\(^{-2}\) s\(^{-1}\)) is the peak photon flux as a function of \( z, \tau \) (fs) is the FWHM pulse duration and \( \Delta x(z) \) (cm) and \( \Delta y(z) \) (cm) are the FWHM beam widths. The FWHM beam width in the \( x \) direction, for example, varies as a function of \( z \) as

\[
\Delta x(z) = \Delta x_0 \sqrt{1 + (z/z_R)^2}, \tag{H5}
\]

where \( \Delta x_0 \) is the beam FWHM at the focus and \( z_R \) (cm) is the Rayleigh range.

The peak photon flux, \( \phi_0(z) \), can be found by integration of \( \phi(x, y, z, t) \) over \( x, y \) and \( t \),

\[
\phi_0(z) = \frac{W \frac{\left(4\ln(2)\right)^{3/2}}{\pi}}{h\nu} \frac{1}{\Delta x(z)\Delta y(z)g\tau} \frac{2\sqrt{2Q}}{gTA(z)}, \tag{H6}
\]

where \( W \) (W) is the average laser or SPDC power and \( h\nu \) (J) is the average energy of an incident photon. The second equality emphasizes that the peak photon flux can be expressed as \( 2\sqrt{2} \) multiplied by the mean photon number \( \mu = Q/g \) (photons per pulse) (where \( Q \) (photons s\(^{-1}\)) is the photon rate incident on the sample) divided by the effective mode area, \( A(z) \) (cm\(^2\)), and the effective pulse duration, \( T = \tau / \sqrt{2\ln(2)} \) (fs). The effective beam area as a function of \( z \) is found through the \( x \) and \( y \) integration of the photon flux

\[
A(z) = \frac{\pi \Delta x(z) \Delta y(z)}{2\ln(2)}. \tag{H7}
\]

Using Eqs. (H2), (H4) and (H6), we can rewrite Eq. (H1) in terms of the laser power

\[
F_C = \sqrt{2} \left( \frac{\ln(2)}{\pi} \right)^{3/2} \frac{\sigma_C n W^2}{\tau g (h\nu)^2} \times \int_{-\lambda/2}^{\lambda/2} \frac{K(z)}{\Delta x(z) \Delta y(z)} dz \int \gamma(\lambda)\Phi(\lambda) d\lambda. \tag{H8}
\]

All but one parameter, \( f(c) \), in Eqs. (H1) and (H2) are known through experiments, simulations and specifications. The parameter \( g \) is specified by the manufacturer, \( K(z) \) is determined through Zemax and experimental verification (described in Appendix E), \( \gamma(\lambda) \) is calculated based on optics’ specifications, \( \Phi(\lambda) \) (except in the case of AF455) and \( \sigma_C \) are known from published measurements, \( n \) is measured and \( \phi(x, y, z, t) \) is measured (\( \Delta x_0, \Delta y_0, z_R \) and \( \tau \) were measured as specified in Appendix B). Table III shows sample specific parameters (\( \sigma_C \) in Table II) and Table IV shows experimental parameters general for all samples. The \( f(c) \) parameter is necessary for the determination of a E2PA cross-section upper limit, thus it was found based on comparison of calculated (using Eq. (H8)) and measured C2PEF (fitted slope) as summarized in Table V.

| Sample    | c (\text{uM}) | \( \Phi \) [Ref.] | \int_{\lambda}^{\lambda} \frac{\gamma(\lambda)\Phi(\lambda)d\lambda}{\int_{\lambda}^{\lambda} \Phi(\lambda)d\lambda} |
|-----------|--------------|-------------------|--------------------------------------------------|
| AF455     | 1100         | N/A               | 0.0515                                            |
| Qdot 605  | 8            | 0.74 ± 0.04       | 0.0285                                            |
| Fluorescein| 1100         | 0.93              | 0.0789                                            |
| Rh6G      | 1500         | 0.90              | 0.0484                                            |
| C153      | 1100         | 0.82 ± 0.04       | 0.0580                                            |
| 9RS       | 390          | 0.66              | 0.0157                                            |

The parameter \( f(c) \) is found to be larger than one for two samples, AF455 and qdot 605. This is physically impossible, however it is probable that there are imperfections in our experimental characterizations. Using qdot 605 as an example of a sample with low fluorescence self-absorption since the concentration is only 8 \( \mu \text{M} \), we expect that \( f(c) \) in this case should be \( \approx 1 \). Since the actual
FIG. 8. Spectral overlap summary for two-photon excited fluorescence (2PEF) measurements (a)-(f) of samples (a) AF455 in toluene, (b) qdot 605 in borate buffer, (c) fluorescein in pH 11 water, (d) 9R-S in chloroform, (e) Rh6G in methanol and (f) C153 in toluene and one-photon excited fluorescence (1PEF) collection efficiency measurements (g)-(h) of samples (g) Rh6G in ethanol and (h) fluorescein in pH 11 water. For both C2PEF and E2PEF, the laser excitation (red), fluorophore emission (Em) (magenta), PMT quantum efficiency (QE) (blue), bandpass (BP) filter (light green) and shortpass (SP) filter (orange) spectra are shown. The laser spectrum was measured using a USB4000 OceanOptics spectrometer. The SPDC spectrum is shown in Fig. 5. For 1PEF, the laser excitation (indigo), fluorophore absorption (Abs) (light blue) and emission (Em) (magenta), PMT QE (blue) and BP filter (light green) spectra are shown. The PMT QE is indicated along the left vertical axis, whereas all other spectra use the right vertical axis. For the filters, the right vertical axis indicates the filter optical density (OD), whereas for all other spectra, the right vertical axis shows a relative intensity. The relative intensities of the laser, absorption and emission are normalized to the height of the peak filter OD for the respective plot. The absorption spectra were measured using a spectrophotometer and emission spectra are measured using a fluorometer, except for qdot 605 (data taken from ThermoFisher). The PMT QE was taken from Hamamatsu specifications. All filter spectra are from the manufacturer, except for the SP filter in the 350-550 nm range (we measured in a spectrophotometer).
TABLE IV. Summary of experimental parameters

| Parameter   | unit   | Laser | SPDC |
|-------------|--------|-------|------|
| $\Delta x_0$ | $\mu$m | 49    | 51   |
| $\Delta y_0$ | $\mu$m | 49    | 84   |
| $z_R$       | mm     | 5.1   | 0.4  |
| $\tau$     | fs     | 111   | 1040 |
| $g$         | MHz    | 80    |      |
| $K(z)$      |        |       |      |
| $Q$         | photons s$^{-1}$ | N/A | 9.5x10$^9$ |
| $\mathcal{T}$ |        |       | 0.76 |

$F_{\text{LB}}$ (cnt s$^{-1}$) = 0.22

TABLE V. Comparison of C2PA fit and calculation, determination of $f(c)$

| Sample    | $\frac{f_c}{W}$ fit (cnt s$^{-1}$ $\mu$W$^{-2}$) | $\frac{f_c}{W}$ calc (cnt s$^{-1}$ $\mu$W$^{-2}$) | $f(c)$ |
|-----------|-----------------------------------------------|-----------------------------------------------|--------|
| AF455     | 77.6                                          | 67.0                                          | 1.21   |
| Qdot 605  | 24.0                                          | 15.8                                          | 1.71   |
| Fluorescein| 3.30                                          | 5.90                                          | 0.63   |
| Rb6G      | 10.2                                          | 18.3                                          | 0.65   |
| C153      | 2.17                                          | 2.73                                          | 0.79   |
| 9RS       | 0.271                                         | 0.393                                         | 0.78   |

$^a$ This derived value is $f(c) \times \frac{\lambda_f}{\lambda_i} \int \Phi(\lambda) d\lambda$

value is 1.71, we think our characterizations differ by a factor of $\approx 1.71$ from the actual conditions. For AF455, Table V shows the derived quantum yield multiplied by the fluorescence self-absorption efficiency.

If we assume the expected E2PEF signal depends only linearly on photon flux, we can estimate the E2PEF signal, $F_E$ (cnt s$^{-1}$) as

$$F_E = N_E g f(c) \int_{z_R}^{z_{R+}} K(z) d\lambda \int \gamma(\lambda) \Phi(\lambda) d\lambda,$$  (H9)

where $N_E$ (fluorophores cm$^{-1}$) is the number of fluorophores excited per infinitesimal length $dz$ per laser pulse, defined as

$$N_E = \frac{1}{2} \sigma_E \mathcal{T} \frac{Q}{g} n,$$  (H10)

where $\sigma_E$ (cm$^2$) is the E2PA cross-section and $\mathcal{T}$ is the transmittance of the photons through all of the optics between the center of the crystal and the center of the sample. The parameter $\mathcal{T}$ is included in $N_E$ but not $N_C$ because of the result found in Appendix C where in this Appendix, $\phi$ and $Q$ are implied to be the value at the sample. As we mentioned in Section III and Appendix E, the dependence of the E2PA excitation rate on the spatial overlap of photons is contained in the cross-section (unlike for C2PA) and thus a cross-section is only valid for a beam of constant entanglement area and thus size. Our SPDC beam is not collimated, instead we attempt to compensate for the changing entanglement area by setting the limits of the $z$ integral from $-z_R$ to $z_R$, which is the region that we expect the majority of a potential E2PEF signal to arise from and should have fairly uniform entanglement area and time. We can define $Q$ in terms of the photon flux $\phi(x, y, z, t)$,

$$Q = g \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \phi(x, y, z, t) dx dy dt = \frac{W}{h\nu}. \quad (H11)$$

Here we have arbitrarily chosen to use the photon flux at $z = 0$.

To place an upper bound on the E2PA cross-section we replace $F_E$ in Eq. (H9) with the lower bound of detectable signal, $F_{\text{LB}}$ (cnt s$^{-1}$), we can solve for $\sigma_E$ which becomes the cross-section upper bound, $\sigma_E^{UB}$ (cm$^2$),

$$\sigma_E^{UB} = \frac{2F_{\text{LB}}}{\mathcal{T} Q nf(c) \int_{z_R}^{z_{R+}} K(z) d\lambda \int \gamma(\lambda) \Phi(\lambda) d\lambda}.$$  (H12)

$F_{\text{LB}}$ is defined as two sigma from zero as described in Appendix F, and the parameters $g$, $f(c)$, $K(z)$, $\gamma(\lambda)$, $\Phi(\lambda)$, $Q$ and $n$ are found in the methods described above. All parameters are listed in Table IIIIV and V. In order to generate a curve for E2PEF as a function of the mean photon number as shown along the diagonals in Fig. 3, the slope, $\frac{F_E}{Qf/g}$, is solved for in Eq. (H9) using a given $\sigma_E$. This slope can be multiplied by the mean photon number on the plot to give a generated E2PEF curve.

The uncertainty on our cross-section upper bounds were calculated by propagating the errors in all of the measured and calculated parameters which go into Eq. (H12).

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