

# On two-photon fluorescence microscopy with conventional and entangled photons

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## Abstract

Modern photonic tools allow to generate non-classical light whose properties require quantum description in terms of photon states. The most prominent example are the highly correlated, so-called “entangled”, photon pairs produced by a process known as parametric down-conversion. Among the proposed applications of the non-classical light is entangled photon microscopy. What are the perspectives of this novel technique and how does it compare with conventional two-photon microscopy with classical light? The present contribution addresses these questions using simple conceptual model of two-photon excitation and elementary two-photon counting statistics.

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## I. INTRODUCTION

One of the scientific achievements of the last century is the emergence of new types of microscopy that greatly helped to improve our understanding of the biological micro-world. Combining advanced optoelectronic technology, in particular lasers and sensitive photon detectors, with advanced chemistry and biochemistry of luminescent markers, techniques have been developed that not only allow to discern structures, but also to follow processes inside a living tissue or in a single cell. A prominent example is confocal laser scanning microscopy (CLSM), which for the first time provided truly three dimensional images at submicron resolution. Today the CLSM-microscope is standard equipment in every biological laboratory, but the development continues. The latest addition is the laser scanning microscopy with two-photon excitation [1–3]. The difference between classical CLSM and two-photon LSM is illustrated in Fig. 1. Classical single-photon excitation of a molecule into a fluorescent

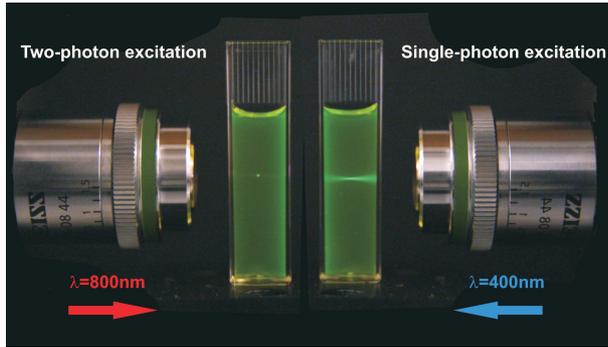


FIG. 1: Excitation profiles with two-photon and one-photon excitation. Picture courtesy M. Kauert.

state involves the absorption of one photon whose energy  $\hbar\omega$  matches the energy difference  $\Delta E_{of} = \hbar\omega_f$  between the ground state  $o$  and an electronic state  $f$  of the molecule. The fluorescence intensity at some position in the exciting laser beam is proportional to the local photon stream, i.e. to the local intensity  $I(\mathbf{r})$  of the beam. The waist like luminescent body that we see in the right sample cell in Fig. 1 is a direct image of  $I(\mathbf{r})$ . In the two-photon case the fluorescence excitation is achieved by the absorption of two photons, whose energies are such that  $\hbar\omega_1 + \hbar\omega_2 = \hbar\omega_f$ . The probability for the two-photon process is proportional to  $I(\mathbf{r})^2$ . The luminescent spot in the left sample cell is the image of this quadratic intensity profile. This is how two-photon LSM achieves the depth resolution, whereas conventional

CLSM requires a spatial filter (e.g. a pinhole) in the observation channel to select the confocal region. Even more important is the following advantage of the two-photon excitation: Each of the excitation events bears a certain risk of damaging the target molecule. For example, the energy absorbed by a dye molecule may trigger an undesired photochemical reaction, instead of being re-emitted as a fluorescence photon. With single photon excitation the damage occurs everywhere in the beam. Thereby, marker molecules are lost without contributing to the signal. Moreover, there is the danger of poisoning the biological sample by aggressive reaction products. With two-photon excitation the collateral damage is restricted to the confocal region. The two-photon process has been predicted 77 years ago by Maria Goeppert-Mayer [4], but two-photon imaging became practicable only in the last decade of the last century, with the advent of femtosecond pulsed lasers. The reason is the low probability of the two-photon process. Efficient two photon excitation requires rather high photon densities which, if applied for prolonged time, would damage the sample simply by heating. With the femtosecond laser the photons are concentrated into intense but rare bunches, allowing experiments at average laser powers that are sufficient for two-photon excitation but harmless for the sample. With this new instrumentation two-photon imaging already became a routine, see e.g. the reviews [2, 3].

Meanwhile, the science of photonics keeps advancing. So far all forms of light microscopy employ the so-called classical light, whose properties are adequately described within electromagnetic wave theory. Today, however, we are able to generate light whose properties require quantum description in terms of photon states. The most prominent example are the highly correlated, so-called “entangled”, photon pairs that can be produced by a process known as parametric down-conversion [5–7]. This process occurs in certain birefringent crystals exhibiting a high non-linear susceptibility (typically  $\beta$ -barium borate BBO). When the illuminating laser beam (called “pump”) is sufficiently strong, then there is a certain probability that an incoming pump photon splits into two photons. Thereby, conservation of energy enforces  $\omega_1 + \omega_2 = \omega_p$ , conservation of momentum requires the matching of the wave vectors  $\mathbf{k}_1 + \mathbf{k}_2 = \mathbf{k}_p$  and conservation of angular momentum puts a corresponding condition on the polarization. Moreover, the two photons share a more or less common time and position of their birth. The structure of the down-converted light field is a rather complex issue depending on the orientation of the crystal with respect to the pump field [5, 6], but one can think of the down-conversion apparatus as producing two light beams. Each of the

beams is like the beam of an ordinary laser beam, randomly producing photons according to Poissonian statistics. However, the photons in the two beams are pairwise correlated: for example, if one detects a photon with energy  $\hbar\omega_1 = \hbar\omega$  in one of the beams, then one is sure to detect  $\hbar\omega_2 = \hbar\omega_p - \hbar\omega$  in the other beam at approximately the same time. Given a stream of such entangled photons, one can perform all sorts of interesting experiments, including such magic topics as “quantum teleportation” [8], “non-locality and Bell inequalities” [9], or even “ghost imaging” [10]. A particularly nice set of not-so-magic but nevertheless interesting and well explained experiments can be found in Ref. [11]. Nearer to practical applicability are “quantum cryptography” [12] and “quantum computation” [13].

Some years ago a new practical application of entangled photons has been proposed, namely “entangled photon microscopy” [14]. The motivation for this proposal seems obvious: The entangled photon twins are generated in nearly the same time and place. When the two beams are recombined and focused into the sample, one automatically obtains “biphotons” ready to participate in two-photon excitation of a molecule. The proposal roused high expectations throughout the imaging community, as expressed for example in Ref. [3]: “(1) Two-photon absorbance is expected to be much better, possibly approaching that of single photon absorption. (2) Power levels required for two-photon excitation may be dramatically reduced, coming close to those for single photon excitation. (3) Spatial resolution would be improved since two-photon excitation would only occur in a volume in which correlated photon pairs overlap in space and time.”

The aims of the present contribution are twofold: One aim is to offer a simple description of the two-photon excitation in terms of elementary photon statistics. This will be done in the spirit of Refs. [14, 15], including, however, certain features of a real experiment. The second aim is to assess the chances for the practicability of entangled photon microscopy. For this assessment, we shall directly compare the performance of three types of two-photon fluorescence experiments, namely i) CW laser excitation, ii) pulsed laser excitation, iii) excitation with entangled photons.

## II. A SIMPLE MODEL

For the purpose of a comparison it is sufficient to adopt a simple model of the two-photon transition, such as has been proposed, e.g., in [14–16]: The exciting light field is quantified in

terms of a probabilistic photon model and the two-photon transition of a molecule is modeled as a successive absorption of two photons. The parameters of the model are compiled in Fig. 2. A first photon induces the transition from the ground state  $o$  to an intermediate state

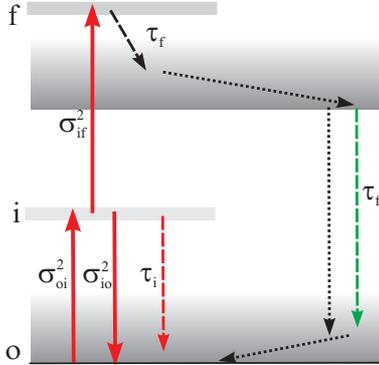


FIG. 2: The two-photon fluorescence model and its parameters. Solid lines: induced transitions. Dashed lines: spontaneous transitions. Dotted lines: non-radiative transitions and transitions through vibrational states.

$i$ , the transition probability being characterized by the cross-section  $\sigma_{oi}^2$ . This  $i$  state exhibits a short but finite lifetime  $\tau_i$ , which reflects “detuning” of the intermediate state with respect to  $\omega_1$  or  $\omega_2$  [15, 17]. A second photon, if it occurs within the lifetime  $\tau_i$ , may complete the two-photon transition, bringing the molecule from  $i$  into a final state  $f$ . The associated cross-section is  $\sigma_{if}^2$ . The second photon may instead induce a downward transition  $i \rightarrow o$ , but this contribution turns out to be negligible, see Appendix A. If none of these events happens, then the molecule returns from  $i$  to  $o$  spontaneously, after spending on average the time  $\tau_i$  in the intermediate state. If the two-photon transition into  $f$  is achieved, then the molecule relaxes rapidly within a short lifetime  $\tau_f$ , cascading thereafter through a series of vibrational states into a long lived state, which may eventually produce a fluorescence photon within a lifetime  $\tau_{fl}$ . The intermediate state  $i$  may be some “real” state, such as a vibronic state of the molecule, or a “virtual state”, for example a linear combination of  $o$  and  $f$ .

Note that we carefully avoid defining the two-photon transition as the “simultaneous absorption of two photons”, which would be questionable in view of the uncertainty relation. Therefore we do not distinguish between “one-step” and “two-step” two-photon transitions [18]. Moreover, we note that in the context of two-photon imaging we are not dealing

with isolated atoms or simple molecules in the gas phase, but rather with a heterogeneous ensemble of dye molecules in solution. We are not interested in exact calculations of two-photon spectra, since the molecular states are anyway disturbed by interactions with the solvent. For any pair of photons with frequencies  $\omega_1$  and  $\omega_2$  there is somewhere a molecule with an available state  $f$  such that  $\omega_f = \omega_1 + \omega_2$ . The heterogeneity of the molecular ensemble is the main justification for the simple probabilistic model: ensemble averaging is likely to smear out quantum interference effects.

The model molecule is exposed to certain intensity  $I = J/A$ , where  $J$  is the optical power of an excitation beam (in photon/s) and  $A$  the cross-section area of the beam. Within the photon model the terms “power” and “intensity” are to be understood as quantum expectation values: If we would measure repeatedly, but with the same power  $J$ , the number  $n_{dt}$  of photons generated by the source in a short time interval  $dt$ , we would obtain a different outcome in each trial, but on average we would get  $\langle n \rangle = Jdt$  photons. At the same time  $Jdt$  can be interpreted as the estimate of the probability to detect a photon within  $dt$ . This is because the time interval  $dt$  is so short that the outcome of a measurement is mostly 0 and only extremely rarely 1. Likewise,  $I dx dy dt$  is the expectation of the number of photons that would be detected with a detector of a small cross-section  $dx dy$  in a short time interval  $dt$ , or the estimate of the probability that a photon hits the target of an area  $dx dy$  within time interval  $dt$ .

With these notions in mind, we can understand the target molecule as a kind of coincidence counter, a molecular version of the classical entangled photon coincidence experiment [19]. The counter consists of two detectors. The first detector of an area  $\sigma_{oi}^2$  represents the transition  $o \rightarrow i$ . If this detector receives a photon then it opens a second detector with a cross-section  $\sigma_{if}^2$ , which represents the transition  $i \rightarrow f$ . The  $if$ -detector remains open during a short time window  $\tau$ , which is terminated by a spontaneous transition  $i \rightarrow o$ . With this tandem detector we make a *gedanken* experiment: We open the  $oi$ -detector for a short time  $dt$  and record the counting values of the two detectors. With  $n_{oi}$  we denote the number of photons counted by the first detector. Since the time interval  $dt$  is infinitesimally short,  $n_{oi}$  can be either 0 or 1, mostly 0 and rarely 1. The value  $c$  recorded by the coincidence detector  $if$  depends on  $n_{oi}$ : if  $n_{oi} = 0$  then  $c = 0$ . If  $n_{oi} = 1$  then the  $if$ -detector starts counting in the interval  $\tau$ , which is assumed to be so short that at most 1 photon is detected. Note that because of this binary logic of the detection process, we can set  $c = n_{oi}n_{if}$ , where  $n_{if}$  is the

counting value one would get from the *if*-detector if it would be opened disregarding the outcome  $n_{oi}$ . After a successful trial the coincidence counter is “dead” until a spontaneous transition brings it back into the ground state, emitting thereby a fluorescence photon. In order to avoid saturation, we must wait at least a couple of fluorescence lifetimes  $\tau_{fl}$  before attempting a new experiment.

Repeating the *gedanken* experiment many times, we can estimate two probabilities,  $dP_{oi}$  and  $dP_{oi,if}$ . The symbol  $d$  in  $dP_{oi}$  is a reminder of the infinitesimally short time interval  $dt$ . The first estimate is the probability  $dP_{oi} = \langle n_{oi} \rangle$  that *oi* detects a photon in the time interval  $dt$ :

$$dP_{oi} = \sigma_{oi}^2 I dt = \frac{\sigma_{oi}^2}{A} J dt \quad (1)$$

The rightmost part of Eq. 1 can be interpreted as follows:  $Jdt$  is the probability that the light source provides at all a photon in the time interval  $dt$  at  $t$ . The ratio  $\sigma_{oi}^2/A$  is the geometrical probability that this photon, traveling somewhere in the beam of a cross-section  $A$ , “hits the target” of a cross-section  $\sigma_{oi}^2$ . The second probability estimate,  $dP_{oi,if}$ , is the one we need in the context of two-photon excitation:  $dP_{oi,if} = \langle n_{oi}n_{if} \rangle$  is the *joint probability* to detect a photon in  $dt$  together with another photon in the interval  $\tau$  adjacent to  $dt$ . In a conventional light source of constant intensity  $I$  the photon arrivals in two different time intervals are uncorrelated and therefore the quantum average  $\langle n_{oi}n_{if} \rangle$  can be factored to  $\langle n_{oi} \rangle \langle n_{if} \rangle$ . Replacing for simplicity the random time window  $\tau$  with its average  $\tau_i$  we obtain:

$$dP_{oi,if} = \sigma_2 I^2 dt = \frac{\sigma_{if}^2}{A} J \tau_i \frac{\sigma_{oi}^2}{A} J dt \quad (2)$$

The middle part of Eq. 2 is the usual expression for the two-photon excitation probability, where  $\sigma_2 = \sigma_{oi}^2 \sigma_{if}^2 \tau_i$  is the so-called two-photon excitation “cross-section” [27], usually given in the unit ”Goepert-Meier”, 1GM= $10^{-50}$ cm<sup>4</sup>s. In the rightmost part of Eq. 2 we arranged the terms to indicate the probabilities of the chain of events: after the first photon has come ( $Jdt$ ) and hit its target ( $\sigma_{oi}^2/A$ ), the second detector triggers:  $J\tau_i$  is the mean number of photons provided by the source within  $\tau_i$ , or the probability to get a photon at all, if  $\tau_i$  is sufficiently short. Finally  $\sigma_{if}^2/A$  is the probability that this second photon hits its target as well. The factor  $\sigma_{oi}^2 \sigma_{if}^2 / A^2$  in Eq. 2 explains why two photon absorption is such a rare process. Typical molecular cross-section are in the order of  $10^{-17}$ cm<sup>2</sup>, whereas the diffraction limited beam cross-section obtained with a high aperture objective is in the order

of  $10^{-9}\text{cm}^2$ . With two-photon excitation, the very small number  $\sigma^2/A$  is squared.

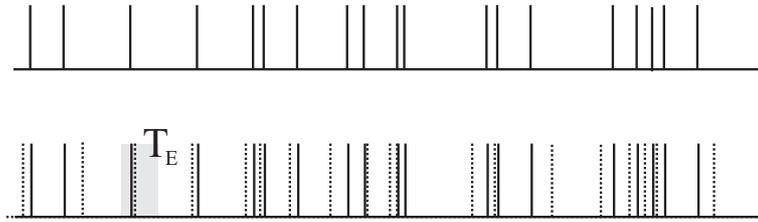


FIG. 3: Upper part: train of photon events from a conventional CW source. Lower part: CW source of entangled photons, dashed lines indicate the twins. The shaded rectangle indicates the distribution  $\epsilon(t + \tau)$  of their arrival times.  $T_E$  is the entanglement time.

The difference between conventional and entangled excitation is that the factoring of the joint quantum probability  $\langle n_{oi}n_{if} \rangle$  is no longer allowed. In fact, this non-factorability defines what is called “non-classical light”. The pairs of entangled photons are correlated in space and in time, due to their twin birth in nearly the same place and time instant. Consequently, these pairs, when re-focused into the sample may arrive at approximately the same time in the same place, which is expected to increase the chance of the twins to hit the same target, and thus achieve the two-photon transition. Indeed, if the two twin photons were perfectly focused into the same point on the target molecule and their arrivals were perfectly timed into  $\tau_i$ , then the  $if$ -detector would trigger as soon as  $oi$  triggers with a probability of 1. In such an ideal situation we would expect  $\langle n_{oi}n_{if} \rangle = \langle n_{oi} \rangle$ . However, this perfect localization is entirely unrealistic, because of the wave nature of the light [19]. Firstly, there is dispersion of the optical path, which will spread the twin arrivals into a time interval  $T_E$ , called entanglement time (see Fig. 3). If  $T_E$  is larger than the time window  $\tau_i$ , then this spreading reduces the hitting probability by a factor  $\tau_i/T_E$ , which reduces  $\langle n_{oi}n_{if} \rangle$  to  $\langle n_{oi} \rangle \tau_i/T_E$ . Secondly, there is the inevitable diffraction, which will spread the twin’s common focus into a finite correlation area  $A_E$ , called entanglement area.  $A_E$  is likely to be of the same order of magnitude as the diffraction limited focus  $A$  of the excitation beam, i.e. much larger than the cross-section  $\sigma_{if}^2$ . The consequence is an additional drastic reduction of the hitting probability by a factor  $\sigma_{if}^2/A$ . On the other hand, since most of the twins keep missing the target, two uncorrelated photons from different entangled pairs get their chance to participate in a two-photon excitation. Therefore the uncorrelated contribution  $\langle n_{oi} \rangle \langle n_{if} \rangle$  will re-emerge. In such a situation the joint counting statistics is tedious (see e.g. [20]), but

the following expression, first order in  $(\sigma_{if}^2/A_E)(\tau_i/T_E)$ , is a very good approximation:

$$\begin{aligned} dP_{oi,if} &= \frac{\sigma_{if}^2}{A_E} \frac{\tau_i}{T_E} \langle n_{oi} \rangle + \langle n_{if} \rangle \langle n_{oi} \rangle \\ &= \left\{ \frac{\sigma_{if}^2}{A_E} \frac{\tau_i}{T_E} + \frac{\sigma_{if}^2}{A} J \tau_i \right\} \frac{\sigma_{oi}^2}{A} J dt = \mathcal{P}_{if|oi} dP_{oi} \end{aligned} \quad (3)$$

In the right most part we introduced for latter convenience the notation of the *joint probability*  $dP_{oi,if}$  in terms of the *conditional probability*  $\mathcal{P}_{if|oi}$  for the excitation  $i \rightarrow f$  under the condition that there was the transition  $o \rightarrow i$ . Equation 3 reveals the essential feature of the entangled two-photon excitation: the first term in curly brackets i.e. the entangled contribution to  $\mathcal{P}_{if|oi}$ , does not depend on the power  $J$ . Therefore, whereas the uncorrelated contribution is proportional to  $J^2$ , the entangled two-photon excitation is linear in  $J$ , as predicted in [21]. Thus, the entangled contribution is expected to dominate at low powers  $J$ , or if  $A \gg A_E$ . However, from the entanglement one gets only at most one photon within  $\tau_i$ , whereas the number of photons  $J\tau_i$  within  $\tau_i$  can be arbitrary large.

### III. FROM GEDANKEN TO REAL EXPERIMENT

In the present section we shall improve the *ad hoc* the reasoning from Sec. II and combine it with the features of a real experiment. Thereby we use the approach from Ref. [22], but neglect the effects associated with the polarization of the excitation and fluorescence, in favor of a more detailed description of the two-photon absorption process. We also neglect the effect of saturation, which was a central topic in [22]. Here we assume that the lifetime  $\tau_{fl}$  of the final fluorescent state is short enough compared with the average interval between two excitation events, so that a given molecule is almost always in its ground state. The sample is not a single molecule but a solution containing the fluorescent molecules at a uniform concentration  $\bar{c}$  molecules/m<sup>3</sup>. The optical power of the source varies with time,  $J(t)$  may represent a train of laser pulses. The photons are carried in a beam focused into the sample. The local intensity  $I(\mathbf{r}, t)$  at a certain position in the sample is proportional to  $J$ , but depends also on the profile of the beam:

$$I(\mathbf{r}, t) = \frac{1}{A} Y(\mathbf{r}) J(t). \quad (4)$$

The dimensionless profile  $Y(\mathbf{r})$  is normalized such that in any cross-section on the propagation axis  $z$  one has  $\int Y(x, y; z) dx dy = A$ , where  $A$  is the effective area of the beam in its focus. A simple example is the Gaussian beam, such as is visualized in Fig. 1:  $Y(\mathbf{r}) = [a_o^2/a^2(z)] \exp[-\rho^2/a^2(z)]$  and  $a^2(z) = a_o^2[1 + z^2/(k^2 a_o^4)]$ ;  $k$  is the wave number. With such a beam we have  $A = \pi a_o^2$ . The entangled exciting light field is actually a superposition of two interfering beams fields with a complex distribution of the spectral content, so that  $Y(\mathbf{r})$  is more complicated. In any case, the strength of fluorescence signal from a volume element of the sample depends on the profile  $Y(\mathbf{r})$ . Neglecting the polarization, the probability to detect a fluorescence photon emitted at  $\mathbf{r}$  can be written as

$$P_D(\mathbf{r}, t) = \eta_D \Phi_o Y_O(\mathbf{r}), \quad (5)$$

where  $\Phi_o = \Omega_o/4\pi$  is the fractional solid angle of the observation,  $\eta_D$  the efficiency of the detection channel and  $Y_o(\mathbf{r})$  the observation profile accounting for the possibility of the selection of the observed region, e.g. with a confocal pinhole. As the measure of the performance of the experiment we take the time averaged count rate  $\bar{J}_F$  of the fluorescence photons, expressed as a function of the time averaged beam power  $\bar{J}$  in photons/s. Thus, the task is to calculate  $\bar{J}_F$  for the three excitation schemes we wish to compare.

So far we were concerned with quantum *gedanken* experiments, repeating measurements with a single molecule exposed to constant intensity  $I$ . In the course of a real experiment, the excitation-detection trial is repeated independently in a vast number of intervals  $dt$  during the continuing time  $t$  at varying intensity  $I(t, \mathbf{r})$ . But there is also a vast number of volume elements  $d^3r$  of the sample, each containing  $c(\mathbf{r}, t)d^3r$  target molecules. With each molecule we have only one quantum trial at a certain  $I(t, \mathbf{r})$ , but because there are so many of them, we can safely replace the particular outcomes by their expectations. Furthermore we neglect the thermal fluctuations of the positions of the molecules, replacing the microscopic distribution[28]  $c(\mathbf{r}, t)$  with its time average  $\bar{c}$ . In the same spirit we also regard the product of molecular cross-sections  $\sigma_{oi}^2 \sigma_{if}^2$  as an average over many orientations and arrangements of the solvent environment. Using the notation introduced in Eq. 3, we write the expected total number of successful trials in the whole experiment as

$$N_F = \bar{c}q \int_T \int_V P_D(\mathbf{r}) \mathcal{P}_{if|oi}(t, \mathbf{r}) dP_{oi}(t, \mathbf{r}) d^3r, \quad (6)$$

from where we obtain the desired photon count rate as  $\bar{J}_F = N_F/T$ . Note that the joint probability  $dP_{oi,if} = \langle n_{oi} n_{if} \rangle = \mathcal{P}_{if|oi} dP_{oi}$  now depends on  $t$  and  $\mathbf{r}$ , reflecting the space and

time profile of the excitation intensity  $I(\mathbf{r}, t)$ :

$$dP_{oi}(t, \mathbf{r}) = \frac{\sigma_{oi}^2}{A} Y(\mathbf{r}) J(t) dt \quad (7)$$

The simple Eq. 3 for the conditional transition probability  $\mathcal{P}_{if|oi}$  may capture the essential features of the two-photon excitation, but there were too many explicit or implicit simplifications involved. Therefore we employ now a more sophisticated version from Appendix A. In the weak perturbation limit, i.e. when the two-photon excitation is a rare process,  $\mathcal{P}_{if|oi}(\mathbf{r}, t)$  can be written as the sum of two contributions  $\mathcal{P}_{if|oi}(\mathbf{r}, t) = \mathcal{P}_{if|oi}^{(u)}(\mathbf{r}, t) + \mathcal{P}_{if|oi}^{(e)}(\mathbf{r}, t)$ , just as in Eq. 3. The uncorrelated contribution  $\mathcal{P}_{if|oi}^{(u)}$  is

$$\mathcal{P}_{if|oi}^{(u)}(\mathbf{r}, t) = \frac{\sigma_{if}^2}{A} Y(\mathbf{r}) \int_0^{T-t} \exp\left[-\frac{\tau}{\tau_i}\right] J(t + \tau) d\tau. \quad (8)$$

Here the averaging of the length of the time window of the *if*-detector is made explicit by the integration over  $\tau$ . The weak perturbation approximation is justified if  $\bar{n}_{\tau_i} \ll A/\sigma_{if}^2$ , where  $\bar{n}_{\tau_i} = \int_0^{\tau_i} J(t + \tau) d\tau$  is the expected number of photons arriving within the intermediate state lifetime  $\tau_i$ . Multiplying  $\mathcal{P}_{if|oi}^{(u)}(\mathbf{r}, t)$  from Eq. 8 with  $dP_{oi}(t, \mathbf{r})$  from Eq. 7, one finds that the fluorescence contribution from a volume element at  $\mathbf{r}$  is proportional to  $|Y(\mathbf{r})|^2$ . This quadratic dependence is the origin of the depth resolution in two-photon scanning microscopy. The expression for the entangled contribution  $\mathcal{P}_{if|oi}^{(e)}(\mathbf{r}, t)$  is assumed to have the same form:

$$\mathcal{P}_{if|oi}^{(e)}(\mathbf{r}, t) = \frac{\sigma_{if}^2}{A_E} Y_E(\mathbf{r}) \int_0^{T-t} \exp\left[-\frac{\tau}{\tau_i}\right] \epsilon(t + \tau) d\tau. \quad (9)$$

Here, the beam profile  $Y(\mathbf{r})/A$  is replaced by the entanglement profile  $Y_E(\mathbf{r})/A_E$  and the beam power  $J(t + \tau)$  is replaced by the distribution  $\epsilon$  of the arrival times of the twins around  $t$ . The distribution  $\epsilon(\tau)$  is normalized so that  $\int_{-\infty}^{+\infty} \epsilon(\tau) = 1$ ; its width defines the entanglement time  $T_E$ . Note that the factoring of the twin correlation into space and time is an approximation introduced to simplify the computation. In general,  $\epsilon$  may also depend on the position  $\mathbf{r}$  in the sample. This would offer the possibility of depth selection by “time focusing”, limited of course by time-frequency uncertainty relation. On the other hand, Ref. [24] suggests that the entangled two-photon excitation profile is essentially the same as the single-photon excitation profile at the pump frequency. This would mean the loss of the depth selection by the quadratic profile  $|Y(\mathbf{r})|^2$ . Lacking a comprehensive theory of the space-time correlation of the entangled photons, as well as experimental data, we resort to the factorization *ansatz* in Eq. 9. Furthermore, in order to shorten the notation, we assume

that  $Y_E(\mathbf{r})/A_E \approx Y(\mathbf{r})/A$ . This allows us to write the combined excitation probability  $\mathcal{P}_{if|oi}(\mathbf{r}, t)$  concisely as

$$\mathcal{P}_{if|oi}(\mathbf{r}, t) \approx \frac{\sigma_{if}^2}{A} Y(\mathbf{r}) \int_0^{T-t} \exp\left[-\frac{\tau}{\tau_i}\right] K(t+\tau) d\tau, \quad (10)$$

where  $K(t+\tau) = J(t+\tau) + \epsilon(t+\tau)$ . The function  $K(t+\tau)$  is analogous to  $J(t+\tau)$ . However,  $K(t+\tau)d\tau$  represents the expected number of photons traversing the beam cross-section within the infinitesimal time interval  $d\tau$  at  $t+\tau$  *under the condition* that there was a photon between  $t$  and  $t+dt$ .

Upon inserting the excitation probability  $\mathcal{P}_{if|oi}(\mathbf{r}, t)$  from Eq. 10, into Eq. 6 we symbolically integrate over  $\mathbf{r}$ . The result of this integration is the overlap integral  $V_O = \int Y^2(\mathbf{r})Y_O(\mathbf{r})d^3r$ , which represents the effective volume of the illuminated and observed region. For example, for a Gaussian beam and unrestricted observation  $V_O = k(\pi a_o^2)^2/2$ , [22]. Note that this  $V_O$  is proportional to  $A^2 = (\pi a_o^2)^2$ , which compensates the corresponding factor in  $\sigma_{oi}^2\sigma_{if}^2/A^2$ . Remarkably, the expected signal does not depend on the focusing of the excitation, as long as the depth of the sample is larger than the Rayleigh parameter  $ka_o^2$  of the exciting beam. Subsequently we shall use the abbreviation  $C = \eta_D q \Phi_O \bar{c} V_O / A^2$ .

What remains to be done is the time integration in Eq. 6, i.e. the averaging over the measuring time  $T$ . Using the obvious assumption that  $T \gg \tau_i$ , we obtain the following general result for  $\bar{J}_F = N_F/T$ :

$$\bar{J}_F(t) = C \sigma_{oi}^2 \sigma_{if}^2 \int_{\tau=0}^{\infty} \exp[-\tau/\tau_i] \langle J(t)K(t+\tau) \rangle_t d\tau, \quad (11)$$

where

$$\langle J(t)K(t+\tau) \rangle_t = \frac{1}{T} \int_{t=0}^T J(t)K(t+\tau) dt. \quad (12)$$

Equation 11 expresses the signal in terms of the correlation function of the expectation values of the quantum photon streams. A rigorous quantum perturbation theory of two-photon absorption in terms of quantum field correlation functions has been worked out long time ago by Mollow [17]. (An intermediate between [17] and the present simplification is Ref. [21].) Our simple probabilistic expression, Eq. 11 is essentially identical with the rigorous result, at least if one is allowed to expect that  $\omega_f = \omega_1 + \omega_2$ ; viz. Eq. 3.17b in [17]. Of course, with our probabilistic model we may miss certain interference effects, such as reported e.g. in [16], but those are anyway likely to be lost by averaging over the heterogeneous ensemble of molecules.

Equation 11 is valid for all three types of experiments; what differs is the correlation function  $\langle J(t)K(t+\tau) \rangle_t$ . For the conventional CW excitation we set  $K(t+\tau) = J(t) = \bar{J}$ . The result of the  $t, \tau$  integrations is the well known quadratic dependence of  $\bar{J}_F$  on the excitation power:

$$\bar{J}_F = C\sigma_{oi}^2\sigma_{if}^2\tau_i\bar{J}^2 = C\sigma_{oi}^2\sigma_{if}^2\bar{n}_i\bar{J}. \quad (13)$$

The second part of Eq. 13 makes the following feature visible: the efficiency  $\bar{J}_F/\bar{J}$ , i.e. the mean number of detected photons per one impinging photon, increases with  $\bar{n}_i = \tau_i\bar{J}$ , i.e. with the mean number of photons arriving during the lifetime  $\tau_i$ .

To evaluate the conventional pulsed excitation we would need a model for the correlation function  $\langle J(t)J(t+\tau) \rangle_t$ . Such models are of course available, but we content ourselves with a model-free approximation. We assume that  $\tau_i$  is much shorter than the laser pulse width  $T_L$ , so that we can set  $\exp[-\tau/\tau_i] \approx \tau_i\delta(\tau)$ , where  $\delta(\tau)$  is the Dirac  $\delta$ -function. A simple calculation yields

$$\bar{J}_F = C\sigma_{oi}^2\sigma_{if}^2\frac{\tau_i}{T_L f}\bar{J}^2 = C\sigma_{oi}^2\sigma_{if}^2\bar{n}_i\bar{J} = C\sigma_{oi}^2\sigma_{if}^2\frac{\tau_i}{T_L}\bar{n}_L\bar{J}. \quad (14)$$

where  $\bar{J} = \frac{1}{T}\int_{t=0}^T J(t)dt$  is the mean laser power,  $f = N_L/T$  is the pulse repetition frequency and the pulse width  $T_L$  is defined as

$$T_L = \frac{1}{N_L} \frac{\left| \int_{t=0}^T J(t)dt \right|^2}{\int_{t=0}^T J^2(t)dt}. \quad (15)$$

Comparing Eq. 14 with the CW-case, Eq. 13, the advantage of the pulsed excitation becomes immediately clear: The number of photons per  $\tau_i$  is now  $\bar{n}_i = (\tau_i\bar{J})/(T_L f)$ ; the enhancement factor  $1/(T_L f)$  makes pulsed excitation much more efficient. The second part of Eq. 14 restates this finding in terms of the mean number of photons per laser pulse,  $\bar{n}_L = \bar{J}/f$ . This form is useful for the comparison with the entangled case.

For the entangled contribution we use a simple approximation: the arrival time of the twin photon is uniformly spread in a time interval  $T_E$ , that is  $\epsilon(t) = 1/T_E$  within  $T_E$  and zero otherwise. In the limit  $\tau_i \ll T_E$  we obtain

$$\bar{J}_F = C\sigma_{oi}^2\sigma_{if}^2\frac{\tau_i}{T_E}\bar{J}. \quad (16)$$

Note here two differences with respect to the pulsed excitation: First, the entangled excitation is only linear in  $\bar{J}$ . Nevertheless, the excitation is still proportional to the square of

the excitation profile  $Y(\mathbf{r})$ , preserving thus the depth resolution property. The second and unfortunate difference is that with entangled photons there is only one twin photon arriving within the correlation time  $T_E$ , whereas with pulsed excitation  $\bar{n}_L$  can be arbitrarily large. The only way to increase the efficiency of the entangled excitation is to improve the timing, i.e. to shorten the correlation time  $T_E$ . There are, however, limits. In the limit of perfect timing we set  $\epsilon(t) = \delta(t)$ , which yields

$$\bar{J}_F = C\sigma_{oi}^2\sigma_{if}^2\bar{J} = C\sigma_{oi}^2\sigma_{if}^2\frac{\tau_i}{\tau_i}\bar{J}. \quad (17)$$

Here  $\tau_i$  disappeared. The second part of the expression is only intended for the comparison with Eq. 16: In the limit of perfect timing the correlation time  $T_E$  is replaced by the life time  $\tau_i$  of the intermediate state. Still, there is only one twin photon per  $\tau_i$ .

#### IV. CONCLUSIONS

To conclude, we express the expected signal from the three two-photon experiments in terms of the two-photon excitation "cross-section"  $\sigma_2 = \sigma_{oi}^2\sigma_{if}^2\tau_i$  and the mean excitation power  $\bar{J}$  used in these experiments:

CW excitation:

$$\bar{J}_F = C\sigma_2\bar{J}_{CW}^2. \quad (18)$$

Pulsed excitation with pulse width  $T_L$  and repetition frequency  $f$ :

$$\bar{J}_F = C\sigma_2\frac{1}{T_L f}\bar{J}_{PL}^2. \quad (19)$$

Entangled CW excitation with entanglement time  $T_E$ :

$$\bar{J}_F = C\sigma_2\left[\frac{1}{\tau_i + T_E} + \bar{J}_{EN}\right]\bar{J}_{EN}. \quad (20)$$

Here, the second term in the brackets represents the conventional background, which is always present, even with the entangled excitation. The entanglement contribution, the first term in the bracket, is an *ad hoc* interpolation between 17 and 16;  $\tau_i$  is the life time of the intermediate state. Note that the conventional background dominates as long  $\bar{J}_{EN}(\tau_i + T_E) > 1$ . Assuming, for example, a twin correlation time  $T_E$  of about 100fs so that  $T_E \gg \tau_i$ , the entanglement contribution would begin to emerge above the conventional CW contribution only at photon streams lower than  $10^{13}$  photons/s, i.e. at powers lower than  $1\mu\text{W}$ .

Today, the most frequently used two-photon excitation scheme employs a femtosecond pulsed laser. In the experiments described in Ref. [22] we used an amplified TiS running at a frequency  $f = 250000$  pulses/s and pulse width  $T_L = 1.1$ ps. Under these conditions the enhancement factor  $1/(T_L f)$  is  $3.6 \times 10^6$ . This high enhancement factor made it possible to measure the two-photon excitation "cross-section" of the common dye rhodamine B at a low concentration of  $10^{-6}$ mol/l. Using the power of  $100\mu\text{W}$ , i.e. photon stream  $\bar{J}_{PL} = 0.4 \times 10^{15}$ photons/s, we measured  $\bar{J}_F$  of roughly  $10^4$  photons/s. In order to match this performance with a CW laser, we need the photon stream

$$\bar{J}_{CW} = \sqrt{\frac{1}{T_L f}} \bar{J}_{PL} = 1900 \times \bar{J}_{PL}. \quad (21)$$

Thus, get a count rate of  $10^4$  photons/s we would need a CW power of roughly 200mW, which is quite realistic. On the other hand, in order to match this performance with entangled twins correlated within  $T_E = 100$ fs, we need the photon stream

$$\bar{J}_{EN} = \frac{\tau_i + T_E}{T_L f} \bar{J}_{PL}^2 \approx 1.5 \times 10^8 \bar{J}_{PL} \quad (22)$$

Thus, get a count rate of  $10^4$  photons/s we need an entangled CW power of roughly 15kW! One could object that the comparison is unfair, because in the reference experiment we used an expensive amplified laser, allowing to measure small  $\sigma_2$  at very low concentration. Therefore we turn the reasoning around and assess the entangled case at realistic powers, using thereby the reference experiment for calibration of the pre-factor  $C\sigma_2$ . In the reference experiment we measured  $C\sigma_2 = 1.7 \times 10^{-32}$ s at a dye concentration  $\bar{c} = 10^{-6}$ mol/l and  $\sigma_2 \approx 10 \times 10^{-50}$ cm<sup>4</sup>s. We insert this  $C\sigma_2$  into Eq. 20 using thereby  $T_E = 100$ fs and a high but realistic entangled power  $\bar{J}_{EN} = 0.4 \times 10^{13}$ photons/s corresponding to about  $1\mu\text{W}$ . The result is  $0.7 \times 10^{-6}$ photons/s of the entangled contribution and  $0.3 \times 10^{-6}$ photons/s for the conventional background. In the reference experiment we used a low magnification objective 10x0.35. Thus, one could improve the figure by about a factor 12 by improving the light collection efficiency. We are at about  $10^{-5}$ photons/s. Increasing the power of the entangled light source without correspondingly decreasing the correlation time  $T_E$  would bring nothing, since this would increase over proportionally the conventional contribution  $\propto \bar{J}_{EN}^2$ . Thus, the only way to increase the yield of the entangled two-photon absorption is to use a highly concentrated solution of a dye having a giant two-photon cross-section  $\sigma_2$ , as it has been (possibly) realized in Ref. [25] using a film of porphyrine dendrimers. Or,

one can use a second nonlinear crystal to recombine the twins in an up-conversion process, as proposed in already in Ref. [26]. But this brings us far from the practice of two-photon imaging.

Of course, one can try to perfection the timing of the twin photons. However, the physical plausibility, namely the uncertainty relation, suggest that  $T_E$  can't be shorter than one cycle of the field oscillation. One can hardly expect  $T_E$  shorter than about 1fs. Another way would be to try to concentrate the twin photons into a tiny entanglement area  $A_E$ . However, in order to surpass the performance of a conventional pulsed experiment, the size of  $A_E$  would have to approach the size of the molecular cross-section  $\sigma_{if}^2$ , i.e. many orders of magnitude smaller than  $\lambda^2$ . This seems to be the main problem with many potential practical applications of entangled photons: the molecular cross-sections are tiny, much unlike the macroscopic cross-sections of beam splitters employed in experiments demonstrating the non-classical features of the entangled light field. These findings may explain why the “entangled photon microscopy” still awaits its realization despite almost ten years of research. Perhaps we tend to get a little bit too mystified by the magic of words such as “quantum entanglement”.

## Appendix A

We need the probability  $\mathcal{P}_{if|oi} = P_{i \rightarrow f}$  for the transition from the intermediate state  $i$  to the final state  $f$ . For simplicity we first neglect the induced transition  $i \rightarrow o$ . Because the probabilities for non-occurrence of events are easier to calculate than occurrence probabilities, and because there are only two outcomes for the molecule in state  $i$ , we express  $P_{i \rightarrow f}$  in terms of its complement:

$$P_{i \rightarrow f} = 1 - P_{i \rightarrow o} \quad (23)$$

Here  $P_{i \rightarrow o}$  represents the probability that the molecule returns from  $i$  to  $o$  without ever reaching  $f$ . Obviously, this return can occur at any time after the excitation at  $t = 0$ , so that  $P_{i \rightarrow o}$  is in the form

$$P_{i \rightarrow o} = \int_{\theta=0}^{\infty} S(\theta) \frac{1}{\tau_i} d\theta. \quad (24)$$

This expression means the following: In order to return to  $o$  at some particular time  $\theta$ , the molecule must have survived in the intermediate state  $i$  during the whole period  $(0, \theta)$ . Let's call this survival probability  $S(\theta)$ . Having survived until  $\theta$ , the molecule may go into

$o$  within  $d\theta$  with a tiny probability which is proportional to  $d\theta$  and which does not depend on  $\theta$ . Thus the proportionality factor is a constant with the unit 1/time. I call this constant  $1/\tau_i$ , for the obvious reasons. The differential probability for the survival and the return at some  $\theta$  is the product  $dP_{i \rightarrow o} = S(\theta)d\theta/\tau_i$ .

In order to survive until  $\theta$ , two “non-events” must happen: i) the molecule does not relax from  $i$  into  $o$ ; the corresponding probability be  $S_o$ . ii) the molecule is not excited from  $i$  to  $f$ ; probability  $S_f$ . The two non-events are mutually independent and therefore

$$S(\theta) = S_o(\theta)S_f(\theta) \quad (25)$$

The calculation of  $S_o(\theta)$  is a classical exercise in introductory statistical physics: We subdivide time period  $\theta$  a in a number  $n$  short consecutive intervals  $\Delta t = \theta/n$ . The intervals  $\Delta t$  are so short that  $S(\theta)$  can be taken as constant. In each of the intervals there may be a transition from  $i$  to  $o$  with the probability  $(\theta/n)\tau_i$ . The probability that this does not happen is the complement  $1 - (\theta/n)\tau_i$ . To survive until  $\theta$  the molecule must have independently survived all  $n$  time intervals. By the virtue of the independence,  $S_o(\theta)$  is simply the product of the partial survival probabilities for all  $n$  intervals:

$$S_o(\theta) = \prod_{i=1}^n [1 - \frac{1}{\tau_i} \Delta t] = \prod_{i=1}^n [1 - \frac{1}{\tau_i} \theta/n] \quad (26)$$

Now we go into the infinitesimal limit  $\Delta t \rightarrow dt$  and  $n \rightarrow \infty$ , while keeping  $n\Delta t = \theta$  constant. The result of this procedure is known from introductory mathematics:

$$S_o(\theta) = \exp(-\theta/\tau_i). \quad (27)$$

In the same way one can also calculate  $S_f(\theta)$ , the probability that the molecule is not excited from  $i$  to  $f$  during  $\theta$ . However, in order to practice photon statistics we use a slightly different approach. Consider first the classical light, whose photon stream obeys Poissonian statistics:

$$P(n, \theta) = \frac{\bar{n}^n}{n!} e^{-\bar{n}}, \quad \text{where} \quad \bar{n} = \int_{\tau=0}^{\theta} J(\tau) d\tau \quad (28)$$

The non-event “ not a transition from  $i$  to  $f$ ” can happen in many different ways: either there was no photon in the stream within  $\theta$ , or there was one photon, but it did not hit the target, or there were two photons but both did not hit the target, or..., or... The probability for not hitting the target is  $1 - q_{if}(\mathbf{r})$ . This non-events are mutually exclusive and to we

can sum up the partial probabilities:

$$S_f(\mathbf{r}, \theta) = \sum_{n=0}^{\infty} \frac{(1 - q_{if}(\mathbf{r}))^n \bar{n}^n}{n!} e^{-\bar{n}} = \exp \left[ -q_{if}(\mathbf{r}) \int_{\tau=0}^{\theta} J(\tau) d\tau \right]. \quad (29)$$

In the same way one also gets the contribution from an entangled photon, except that there are only two possibilities: either no photon in the interval  $(0, \theta)$ , or there is one photon in  $(0, \theta)$  but no hit.

Upon collecting the results we obtain  $P_{i \rightarrow o}$  as

$$P_{i \rightarrow o} = \int_0^{\infty} \frac{1}{\tau_i} \exp \left[ -\frac{\theta}{\tau_i} \right] \exp \left[ -q_{if}^{(c)} \mathcal{C}(\theta) - q_{if}^{(e)} \mathcal{E}(\theta) \right] d\theta, \quad (30)$$

$$\mathcal{C}(\theta) = \int_0^{\theta} J(t + \tau) d\tau, \quad \text{and} \quad \mathcal{E}(\theta) = \int_0^{\theta} \epsilon(t + \tau) d\tau. \quad (31)$$

Here  $\epsilon(t + \tau)$  is a distribution describing the spread of the arrival times of the twin around  $t$ .  $\epsilon(\tau)$  is normalized so that  $\int_{-\infty}^{+\infty} \epsilon(\tau) = 1$ . Its width defines the entanglement time  $\tau_C$ . The symbols  $q_{if}(\mathbf{r})$  represent the geometrical probabilities that a photon from the exciting beam hits the target  $\sigma_{oi}^2$ . For conventional photons uncorrelated with the previous transition  $o \rightarrow i$  this probability is  $q_{if}^{(c)}(\mathbf{r}) = (\sigma_{oi}^2/A)Y(\mathbf{r})$ , where  $A$  is the effective cross-section and  $Y(\mathbf{r})$  the profile of the beam. For entangled photons that are correlated with the previous transition  $o \rightarrow i$  this probability is  $q_{if}^{(e)}(\mathbf{r}) = (\sigma_{oi}^2/A_E)Y_E(\mathbf{r})$ , where  $A_E$  is the entanglement area  $Y_E(\mathbf{r})$  the entanglement profile.

To simplify the matters, we assume now that the lifetime  $\tau_i$  of the intermediate state is so short, and/or that the cross-section  $\sigma_{if}^2$  is so small that the argument of the second exponential in Eq. 30 is close to zero in the whole relevant range of  $\theta$ . In other words, we assume that  $\bar{n}_{\tau_i} \ll \pi A^2/\sigma_{if}^2$ . Thus, we expand the exponential up to the first order of the argument, carry out the obvious integration to write directly the probability  $P_{i \rightarrow f}$  for the transition  $i \rightarrow f$ :

$$P_{i \rightarrow f} \approx - \int_0^{\infty} \frac{1}{\tau_i} \exp \left[ -\frac{\theta}{\tau_i} \right] \int_0^{\theta} \left[ q_{if}^{(c)} J_E(t + \tau) + q_{if}^{(e)} \epsilon(t + \tau) \right] d\tau d\theta \quad (32)$$

Integrating by parts we get a rather simple expression

$$P_{i \rightarrow f} \approx \int_0^{\infty} \exp \left[ -\frac{\theta}{\tau_i} \right] \left[ q_{if}^{(c)} J_E(t + \theta) + q_{if}^{(e)} \epsilon(t + \theta) \right] d\theta. \quad (33)$$

When the induced transition  $i \rightarrow o$  is included, then Eq. 30 is modified by the presence of the downward path. The general form is the same for the conventional and for the entangled

case:

$$P_{i \rightarrow o} = \int_0^\infty \left[ \frac{1}{\tau_i} + q_{io}(\mathbf{r})K(t + \theta) \right] \exp \left[ -\frac{\theta}{\tau_i} \right] \exp \left[ -q'(\mathbf{r}) \int_0^\theta K(t + \tau) d\tau \right] d\theta. \quad (34)$$

where  $q_{io}$  is the equivalent of  $q_{if}$  but for the downward transition  $i \rightarrow o$  and  $q'(\mathbf{r}) = q_{if}(\mathbf{r}) + q_{io}(\mathbf{r})$ . After carrying out the somewhat lengthy calculation, one finds that the induced transition subtracts from  $P_{i \rightarrow f}$  a term which is proportional to  $(\bar{n}_{\tau_i} \sigma_{io}^2 / \pi A^2)^2$ . We have already assumed that  $(\bar{n}_{\tau_i} \sigma_{if}^2 / \pi A^2) \ll 1$ , and so we neglect the induced term as well. In other words, we keep assuming that the perturbation of the molecule by the impinging light field is weak, and therefore both photon induced transitions,  $i \rightarrow f$  and  $i \rightarrow o$ , are rare compared with the spontaneous decay of the intermediate state.

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- [1] W. Denk, J.H. Strickler, and W.W. Webb. Two-photon laser scanning fluorescence microscopy. *Science*, 248:73–76, 1990.
  - [2] A. Diaspro, G. Chirico, and M. Collini. Two-photon fluorescence excitation and related techniques in biological microscopy. *Quarterly Reviews of Biophysics*, 38:97–166, 2006.
  - [3] E. Niggli and M. Egger. Applications of multi-photon microscopy in cell physiology. *Frontiers in Bioscience*, 9:1598–1610, 2004.
  - [4] M. Goepfert-Mayer. Ueber elementarakte mit zwei quantenspruengen. *Ann. Phys.*, 9:273–295, 1931.
  - [5] R. Andrews, E. R. Pike, and S. Sarkar. Photon correlations and interference in type-i optical parametric down-conversion. *J. Opt. B: Quantum Semiclass. Opt.*, 1:588–597, 1999.
  - [6] Ch. Kurtsiefer, M. Oberparleiter, and H. Weinfurter. Generation of correlated photon pairs in type-ii parametric down conversion- revisited. *Journal of Modern Optics*, 48:1997–2007, 2001.
  - [7] D. Dehlinger and M. W. Mitchell. Entangled photon apparatus for the undergraduate laboratory. *Am. J. Phys.*, 70:898–902, 2002.
  - [8] D. Bouwmeester, J.W. Pan, K. Mattle, M. Eibl, H. Weinfurter, and A. Zeilinger. Experimental quantum teleportation. *Nature*, 390:575, 1997.
  - [9] D. Dehlinger and M. W. Mitchell. Entangled photons, nonlocality, and bell inequalities in the undergraduate laboratory. *Am. J. Phys.*, 70:903–910, 2002.

- [10] M. Bache, E. Brambilla, A. Gatti, and L.A. Lugiato. Ghost imaging schemes: fast and broadband. *Optics Express*, 12:6067–6081, 2004.
- [11] E. J. Galvez, C. H. Holbrow, M. J. Pysher, J. W. Martin, N. Courtemanche, L. Heilig, and J. Spencer. Interference with correlated photons: Five quantum mechanics experiments for undergraduates. *Am. J. Phys.*, 70:127–140, 2005.
- [12] C. H. Bennett, G. Brassard, and A. K. Ekert. Quantum cryptography. *Scientific American*, October:50–57, 1992.
- [13] C. H. Bennett. Quantum information and computation. *Physics Today*, 48:24–307, 1995.
- [14] M. C. Teich and B. E. A. Saleh. Entangled-photon microscopy. *Ceskoslovensky casopis pro fyziku (Prague)*, 47:3–8, 1997.
- [15] F. A. Moscatelli. A simple conceptual model for two-photon absorption. *Am. J. Phys.*, 54:52–54, 1986.
- [16] H-B. Fei, B. M. Jost, S. Popescu, B. E. A. Saleh, and M. C. Teich. Entanglement-induced two-photon transparency. *Phys. Rev. Lett.*, 78:1679 – 1682, 1997.
- [17] B.R. Mollow. Two-photon absorption and field correlation functions. *Phys. Rev.*, 175:1555–1563, 1968.
- [18] P. Lambropoulos. Theory of multiphoton ionization: Near-resonance effects in two-photon ionization. *Phys. Rev. A*, 9:1992–2013, 1974.
- [19] C.K. Hong and L. Mandel. Experimental realization of a localized one-photon state. *Phys. Rev. Lett.*, 56:58–60, 1986.
- [20] M. M. Hayat, S. N. Torres, and L. M. Pedrotti. Theory of photon coincidence statistics in photon-correlated beams. *Optics Communications*, 169:275–287, 1999.
- [21] J.Javanainen and Ph. L. Gould. Linear intensity dependence of a two-photon transition rate. *Phys. Rev. A*, 41:5088–5091, 1990.
- [22] M. Kauert, P. C. Stoller, M. Frenz, and J.Rička. Absolute measurement of molecular two-photon absorption cross-sections using a fluorescence saturation technique. *Optics Express*, 14:8434–8447, 2007.
- [23] K.M. Berland, P.T. So, and E. Gratton. Two-photon fluorescence correlation spectroscopy: method and application to the intracellular environment. *Biophys J.*, 68:694–701, 1995.
- [24] M.B. Nasr, A.F. Abouraddy, M.C. Booth, B.E. Saleh, A.V. Sergienko, M.C. Teich, C. Malvin, M. Kempe, and R. Wolleschensky. Biphoton focusing for two-photon excitation. *Phys. Rev.*

- A, 65:023816.1–023816.6, 2002.
- [25] D-I. Lee and Th. Goodson III. Entangled photon absorption in an organic porphyrin dendrimer. *J. Phys. Chem. B*, 110:25582–25585, 2006.
- [26] V. M. Petnikova. Nonlinear optical efficiency of two-photon fields. *Sov. J. Quantum Electron.*, 9:276–280, 1979.
- [27] Our definition would occur the rate equation for the concentration  $c_0$  of the absorbing molecules in the ground state, namely on  $dc_0 = -\sigma_2 c_0 I^2 dt$ . Other authors prefer to regard the absorption process from the point of view of the impinging photons, employing a "quadratic Beers-Law":  $dI = -\delta c_0 I^2 dx$ . We see that  $\delta = 2\sigma_2$ , since two photons are absorbed in each excitation event. This unfortunate duality still keeps causing confusion.
- [28] The microscopic distribution  $c(\mathbf{r}, t)$  is defined as  $c(\mathbf{r}, t) = \sum_i \delta(\mathbf{r} - \mathbf{r}_i)$ . The macroscopic concentration  $\bar{c}$  is a time or ensemble average  $\bar{c} = \langle c(\mathbf{r}, t) \rangle$ . The fluctuation of  $c(\mathbf{r}, t)$  are the subject of the technique known as "fluorescence correlation spectroscopy", which is closely related with confocal and two-photon imaging [23].