Homework 2

Nano Optics, Fall Semester 2018
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Due date: November 02, 2018; 10 a.m.

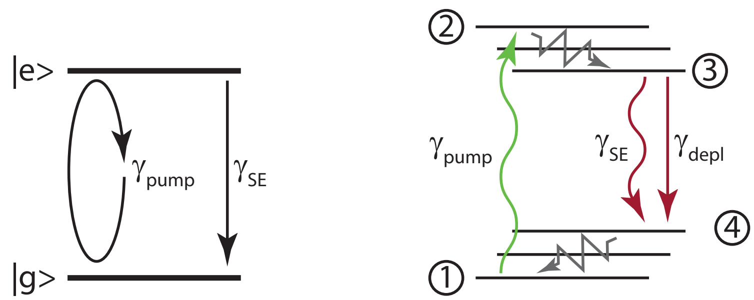
The goal of this homework is to understand the basics of fluorescence microscopy and stimulated emission depletion superresolution microscopy.
Please stick to the following points:

- Hand in your solution on time (beginning of lecture) in legible hand-written form or typeset using LaTeX (do not provide any code of any programming language, do not send your solutions by email).
- Keep your solutions in the order of the problem set.
- Show explicitly how you arrived at your answer and comment on your thoughts. Focus on the presentation of your solutions as if you were to explain them to a reader who is not familiar with the topic. Be brief but to the point.
- If you decide to generate graphs with a computer (which is encouraged), please insert them at the appropriate position within your solution.
- Always label graphs and axes and provide units where appropriate.
- You can receive a maximum of 100 points for the correct solutions to the problems. For the quality of your presentation, you can receive an additional 10 points.

You are strongly encouraged to discuss the problems with your peers. In case of remaining questions, feel free to approach the course instructors.

1 Fluorophores [35 pts.]

Fluorophores are optically active quantum systems, which means that they strongly interact with electromagnetic fields. Simple fluorophores are atoms or molecules, quasi-atoms like defects in crystals, or artificial atoms like semiconductor quantum dots.
To gain a basic understanding of the light-matter interaction of fluorophores, we first consider a two-level system, a paradigmatic example of quantum mechanics. For simplicity, we neglect all quantum-coherent effects in this exercise. The TLS has a ground state $|g\rangle$ and an excited state $|e\rangle$, split in energy by the energy $\hbar\omega_{ge}$. In a fluorophore, these two states would be the two lowest lying electronic excitation. A light field at the transition frequency ω_{ge} couples the two levels at a rate γ_{pump}, leading to excitation transitions from the ground state to the excited state and stimulated emission from the excited to the ground state. Even in the absence of a pump field, the excited state will decay to the ground state at a rate γ_{SE} due to spontaneous emission.



(a) (4 pts.) Formulate a differential equation governing the temporal evolution of the population probability of the excited state p_e of the TLS in the presence of a pump rate γ_{pump} and a spontaneous emission rate γ_{SE}. Do not forget to take stimulated emission into account.

(b) (4 pts.) Determine the steady state population of the excited state $p_e^{\text{ss}} = p_e(t \to \infty)$ in the presence of a pump rate γ_{pump} and a spontaneous emission rate γ_{SE}. Furthermore, determine the limit of the population of the excited state under strong pumping. Explain in a few words, why one says that "a two-level system cannot be inverted".

Since it cannot be inverted, a two-level system is of limited practical use for certain applications. Luckily, most fluorophores are not perfect two-level systems but have a much richer internal structure. As an example, consider the simplest atom, hydrogen, which already features a sophisticated electronic level scheme. One family of frequently used fluorophores are organic molecules with extended π-electron systems. The electronic levels of interest are typically the electronic ground state $|g\rangle$ and the first excited electronic state $|e\rangle$. Both these states are broadened into a band by vibrational excitations (see sketch on the right in figure above). It is essential that relaxation within the vibrational bands happens on a timescale several orders of magnitude faster than spontaneous emission between the electronic states. Therefore, after excitation from state 1 to state 2, the system relaxes down the vibronic ladder to the lower band edge (state 3) practically instantaneously, such that stimulated emission plays no role. After spontaneous emission from state 3 into the vibrational band of the ground state (state 4), the system returns to the starting state 1 via (extremely fast) vibrational transitions. We therefore treat the vibrationally broadened two level system as an effective four-level system.

(c) (4 pts.) Formulate the equation of motion for the population $p_3(t)$ of state 3 in the presence of a pump beam at transition frequency ω_{12} leading to a coupling rate γ_{pump} and a spontaneous emission rate γ_{SE} of state 3.

(d) (4 pts.) Solve the equation of motion for $p_3(t)$ given that the fluorophore is in state 1 at time $t = 0$. Determine the steady state population $p_3^{\mathrm{ss}} = p_3(t \to \infty)$.

(e) (4 pts.) Plot the population $p_3(t)$ obtained in problem (d) as a function of time. Normalize the time axis suitably to a dimensionless quantity, using the characteristic rates of the problem.

(f) (4 pts.) Formulate the equation of motion for the population of state 3 in the presence of a first light field resonant with the transition between levels 1 and 2 (giving rise to a coupling rate γ_{pump}), as well as a beam resonant with the transition between levels 3 and 4, leading to a coupling rate γ_{depl}. Keep in mind that level 3 also decays spontaneously at a rate γ_{SE}. Furthermore, remember that all phononic decay rates largely exceed any optical transition rate.

(g) (3 pts.) Assume that we start the system described in problem (f), but in absence of the pump beam, in state 3 with the initial population $p_3^0 = p_3(t = 0)$. Solve the equation of motion for $p_3(t)$ derived in problem (f).

(h) (4 pts.) Plot the normalized population $p_3(t)/p_3^0$ obtained in problem (g) as a function of time. Normalize the time axis suitably to a dimensionless quantity.

(i) (4 pts.) To conclude, we now consider the rates γ. The pump rate depends on the pump intensity via

$$\gamma_{\mathrm{pump}} = \sigma_{\mathrm{abs}}(\omega_{\mathrm{pump}})\, I_{\mathrm{pump}}/(\hbar\omega_{\mathrm{pump}}), \tag{1}$$

with the absorption cross section σ_{abs} at the pump frequency ω_{pump}. Assume you have a single laser beam carrying a power P, which is resonant with the transition between the levels 1 and 2, focused to a diffraction limited spot centered on the four-level system. At which rate does the fluorophore emit photons at the transition frequency ω_{34}?

Hint: Assume that the focal intensity spot has a Gaussian shape and and full width at 1/e of the maximum of $\lambda/(2\mathrm{NA})$. Furthermore, the fluorophore is small enough compared to the wavelength of light to be considered a mathematical point.

2 Stimulated emission depletion superresolution microscopy [65 pts.]

Since Abbe, microscopy using electromagnetic fields was thought to be limited by diffraction. Abbe himself showed that a monochromatic field of vacuum-wavelength λ can be focused to a spot of characteristic size

$$x_0 = \frac{\lambda}{2\,\mathrm{NA}}. \tag{2}$$

Here, the numerical aperture $\mathrm{NA} = n \sin\alpha$ is defined by the refractive index n of the medium and the opening angle of the focusing optics α. We can get an intuitive idea of the resolution limit by looking at the interference fringes generated by just two plane waves.

(a) (3 pts.) Consider a y-polarized monochromatic plane wave at frequency ω in vacuum with amplitude E_0 whose wave vector $\boldsymbol{k}_1$ lies in the xz-plane and makes an angle θ with the z-axis. Write down the complex spatial amplitude function $\boldsymbol{E}_1(\boldsymbol{r})$ and express the wave vector in terms of the angle θ.

(b) (4 pts.) Calculate the magnetic field $\boldsymbol{H}_1(\boldsymbol{r})$ from $\boldsymbol{E}_1(\boldsymbol{r})$ and express its amplitude using the vacuum impedance $Z_0 = \sqrt{\mu_0/\varepsilon_0}$.

(c) (8 pts.) We now consider a second y-polarized monochromatic plane wave $\boldsymbol{E}_2(\boldsymbol{r})$ of equal amplitude E_0 and frequency ω propagating in the same plane as the first, but under the angle $-\theta$ with respect the the z-axis. Calculate the intensity of the superposition of the two fields in the plane $z = 0$ and sketch it as a function of x, assuming $E_0 \in \mathbb{R}$. Determine the periodicity of the interference pattern as a function of the wavelength λ and the angle θ. What is the maximum intensity you can reach? What is the minimum periodicity you can reach for a given λ?

We just saw that with radiation of wavelength λ we can generate an interference pattern with a minimal characteristic feature size of $\lambda/2$. We reach this limit when forming a standing wave of two counter-propagating plane waves. By interfering an infinite number of plane waves, propagating within the angle $-\alpha \ldots \alpha$ we obtain a single bright spot at the origin with a characteristic size according to Eq. (2).

We now turn to a method able to break the diffraction limit, called stimulated emission depletion (STED), which won its inventor Stefan Hell part of the Nobel Prize in chemistry in 2014. Our goal is to localize fluorescing emitters distributed in the focal plane. For calculational simplicity, instead of assuming lasers focused to single spots, let the intensity of the excitation field be that of a standing wave

$$I_{\text{ex}}(x) = I_0^{\text{ex}} \cos^2(x/x_0), \tag{3}$$

with $x_0 = \lambda/(2\,\mathrm{NA})$. The frequency of the excitation field is chosen to match the transition between levels 1 and 2 in the right level scheme sketched in problem 1. Furthermore, let there be a depletion field, with intensity distribution

$$I_{\text{STED}}(x) = I_0^{\text{STED}} \sin^2(x/x_0), \tag{4}$$

whose frequency is matched to the transition between levels 3 and 4. To keep our discussion simple and to illustrate the idea at the heart of STED, we treat here a time-gated version using pulsed lasers. Countless variants of STED are in use in laboratories around the world.

The idea behind STED is to optically localize fluorescing markers by overlaying a pump beam and a depletion beam such that the depletion beam has a zero where the pump beam has its maximum. A weak excitation pulse pumps the fluorophore to its excited state. The excitation pulse is followed by a strong depletion pulse, forcing all emitters down to the ground state except those located in a very narrow region around the focal center. After arrival of the two pulses, fluorescence photons are collected, which can only stem from emitters located in an area of subwavelength dimension around the focal center.

(d) (4 pts.) Formulate the position dependent probability $p(x)$ of a fluorophore to be in the excited state after an excitation pulse of duration τ_{ex}, assuming the pulse duration is much shorter than the inverse sum of the transition rates γ_{pump} and γ_{SE}. Your expression should only contain the absorption cross section $\sigma_{\text{ex}}(\omega_{\text{ex}})$, the excitation frequency ω_{ex}, the excitation pulse duration τ_{ex}, and the position dependent pump intensity $I_{\text{ex}}(x)$, and Planck's constant $\hbar$. Use your insights from problem 1.

(e) (4 pts.) Right after the excitation pulse, we expose the emitter to a stimulated emission depletion pulse of duration τ_{STED}. Formulate the probability $p_{\text{STED}}(x)$ of the emitter to be in the exited state after the STED pulse under the assumption $\gamma_{\text{STED}} \gg \gamma_{\text{SE}}$. Your expression should contain only the probability p_{ex}, the absorption cross section at the STED frequency σ_{STED}, the STED frequency ω_{STED}, the pulse duration τ_{STED}, the STED intensity $I_{\text{STED}}(x)$, and Planck's constant.

(f) (6 pts.) We are interested in the population $p_{\text{STED}}(x)$ for small x. Develop the field intensities $I_{\text{ex}}(x)$ and $I_{\text{STED}}(x)$ up to terms quadratic in x/x_0.

(g) (6 pts.) Use your approximations for the field intensities to derive an expression for the population after the STED pulse $p_{\text{STED}}(x)$ close to the origin. Introduce the saturation intensities of the excitaton beam $I_{\text{sat}}^{\text{ex}} = \hbar\omega_{\text{ex}}/(\sigma_{\text{ex}}\tau_{\text{ex}})$ and of the STED beam $I_{\text{sat}}^{\text{STED}} = \hbar\omega_{\text{STED}}/(\sigma_{\text{STED}}\tau_{\text{STED}})$ to simplify your notation.

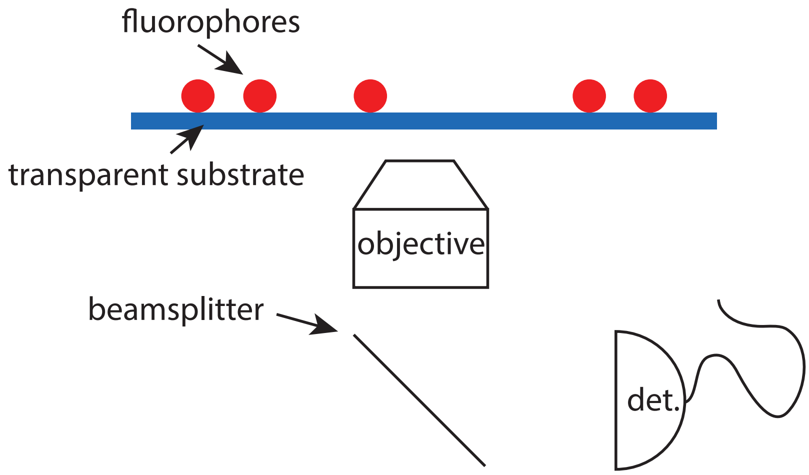
(h) (6 pts.) Show that, to leading order in x/x_0, the full-width at half-maximum of the population distribution $p_{\text{STED}}(x)$ reads

$$\Delta x = A\frac{\Delta}{\sqrt{1+B}}, \tag{5}$$

with Abbe's diffraction limit Δ and a prefactor A of order unity. Accordingly, stimulated emission depletion suppresses the diffraction limit by a factor $1/\sqrt{1+B}$. Determine the factor B, which depends on the intensities I_0^{STED} and $I_{\text{sat}}^{\text{STED}}$.

(i) (10 pts.) Generate a graph where you plot the pump intensity $I_{\text{ex}}(x)$ and the STED intensity $I_{\text{STED}}(x)$ in the range $-\pi/2 < x/x_0 < \pi/2$. Furthermore, plot the population probability $p_{\text{STED}}(x)$ after the STED pulse both under the approximation you made above and with the full numerical result for $I_0^{\text{STED}}/I_{\text{sat}}^{\text{STED}} = 100$. Normalize all quantities appropriately to be dimensionless.

(j) (10 pts.) Based on your understanding of STED microscopy gained by your calculations, complete the sketch below and describe in your own words how you would set up a STED microscope and how you would operate it in order to image a distribution of fluorophores in the focal plane of a microscope objective. Comment on whether it is possible to do STED without using pulsed lasers and without making some temporal selection of the detected photons.



(k) (4 pts.) In your own words, what is at the heart of STED to allow for the breaking of Abbe's diffraction limit.