Sample: Optics - Optics Homework

1. Say you have a solution of AlexaFluor 488 at 30 µM in a tall cuvette that is 5 mm wide and 5 mm deep. AlexaFluor 488 has a fluorescence life time of 4.1 ns and a molar extinction coefficient of 73,000 cm^{-1} M^{-1} at 495 nm.

   A) If you bring a 1 mW 495 nm light at upon the sample, how much power gets transmitted through the sample assuming no reflection or absorption by the cuvette?

   B) If the pulse of light only lasts for 10 ps, how many fluorophores were excited? (Remember we can count the number of photons in this pulse assuming it was exactly 1 mW for 10 ps.) Assume the laser has a 4 mm beam diameter.

   C) If the pulse of 495 nm light is very short in time and we define the moment the 495 nm light transmits through the sample as t= 0, what fraction of the fluorophores that got excited were still excited at t = 3 ns? Draw an approximate graph with well labeled axes of the number of excited fluorophores vs. time.

Solution

a) Using the Beer–Lambert law, we get that the power per area, which transmitted through the cuvette

$$I = I_0 10^{-A}$$

$$A = \varepsilon Cl = \log_{10}\frac{I_0}{I}$$

ε is molar extinction coefficient, l is path of light through the cuvette, C is number of moles of absorbent. Whence, we get

$$\varepsilon = 73000 cm^{-1} M^{-1}$$

$$C = 30\mu M$$

$$l = 5mm = 0.5cm$$

$$A = \varepsilon Cl = 1.095$$

$$I = I_0 \cdot 10^{-1.095} = 0.0803 I_0$$

b) The energy of one photon is $E_{ph} = h\upsilon = h\frac{c}{\lambda}$, where

$$h = 6.626 \cdot 10^{-34} J \cdot s$$

$$c = 3 \cdot 10^8 \frac{m}{s}$$

$$\lambda = 495nm = 4.95 \cdot 10^{-7} m$$

$$E_{ph} = 4.016 \cdot 10^{-19} J$$

The laser emitted the energy

$$E = t \cdot W$$
$$W = 1mW$$
$$t = 1ps$$
$$E = 10^{-15} J$$

Whence, the laser emitted the number of photons

$$N_0 = \frac{E}{E_{ph}} = 2.5 \cdot 10^3$$

The fluorescence medium absorbed only $N = N_0 \frac{I}{I_0} \frac{S}{S_{cuv}}$, where ***S*** is cross-section of laser beam, $\boldsymbol{S_{curv}}$ is cross-sectional of cuvette.

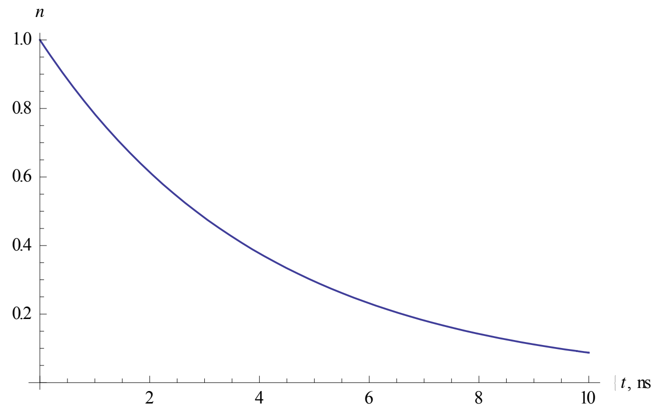
$$d = 4mm$$
$$a = 5mm$$
$$S = \frac{\pi d^2}{4} = 12.56mm^2$$
$$S_{cuv} = a^2 = 25mm^2$$

$$N = 0.0404 N_0 = 101$$

We know, that the number of absorbed photons is equal to the number of excited fluorophores.

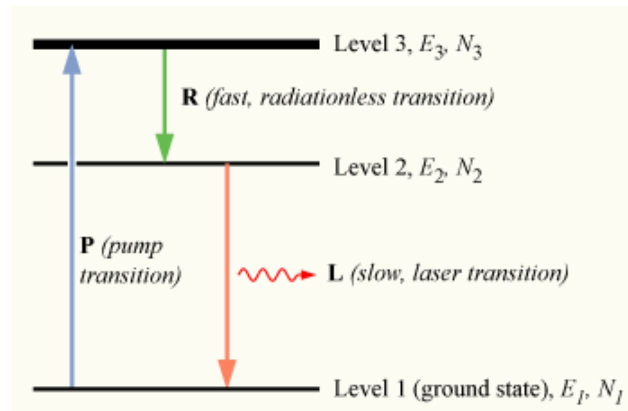
Whence, we get $\boldsymbol{n_0=101}$ excited fluorophores.

c) We know, that the number of fluorophores time dependence is
$$n = n_0 \exp\left(-\frac{t}{t_0}\right)$$
Where t_0 is fluorescence lifetime.
At time ***t=3ns***
$$n = 0.48 n_0 = 48$$



2. Draw the electron energy levels present in a 3-state laser gain medium and indicate the relative relaxation times between each level. Why is it required to have the relaxation times as they are where some are fast and some are slow?

Solution



3-state laser (from http://en.wikipedia.org/wiki/Population_inversion)

If the width of energy level 3 is ΔE_3, the width of energy level 2 ΔE_2, we get from uncertainty principle life-time (relaxation time) at these levels $\Delta t_3, \Delta t_2$

$$\Delta E_3 \Delta t_3 \geq h$$
$$\Delta E_2 \Delta t_2 \geq h$$

$$\Delta t_3 \geq \frac{h}{\Delta E_3}$$
$$\Delta t_2 \geq \frac{h}{\Delta E_2}$$

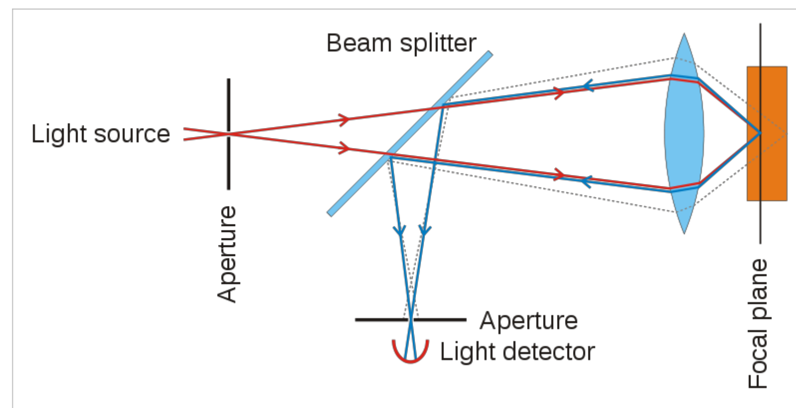
If $\Delta E_3 >> \Delta E_2$ we get $\Delta t_3 << \Delta t_2$

Populations at levels are N_1, N_2, N_3, and total population is N.

Initially, the system of atoms is at thermal equilibrium, and the majority of the atoms will be in the ground state, i.e., $N_1 \approx N$, $N_2 \approx N_3 \approx 0$. If we now subject the atoms to light of a frequency $E_3 - E_1 = h\nu_{13}$), the process of optical absorption will excite the atoms from the ground state to level 3 (pumping).

If we continue pumping the atoms, we will excite an appreciable number of them into level 3, such that $N_3 > 0$. In a medium suitable for laser operation, we require these excited atoms to quickly decay to level 2. The energy released in this transition may be emitted as a photon (spontaneous emission), however in practice the 3→2 transition is usually radiation less, with the energy being transferred to vibrational motion (heat) of the host material surrounding the atoms.
An atom in level 2 may decay by spontaneous emission to the ground state, (releasing a photon of frequency ν_{12}, given by $E_2 - E_1 = h\nu_{12}$). If the lifetime of this transition, Δt_2 is much longer than the lifetime of the radiation less 3 → 2 transition Δt_3 the population of the E_3 will be essentially zero ($N_3 \approx 0$) and a population of excited state atoms will accumulate in level 2 ($N_2 > 0$). If over half the N atoms can be accumulated in this state, this will exceed the population of the ground state N_1. A population inversion ($N_2 > N_1$) has thus been achieved between level 1 and 2, and optical amplification at the frequency ν_{21} can be obtained.

3. Draw a ray diagram and describe the basic principles behind a confocal microscope.



Ray diagram (from)

Confocal microscopy is an optical imaging technique used to increase optical resolution and contrast of a micrograph by using point illumination and a spatial pinhole to eliminate out-of-focus light in specimens that are thicker than the focal plane. It enables the reconstruction of three-dimensional structures from the obtained images.

In the microscope the final image has the same focus as or the focus corresponds to the point of focus in the object. The object and its image are "confocal." The microscope is able to filter out the out-of-focus light from above and below the point of focus in the object. Normally when an object is imaged in the fluorescence microscope, the signal produced is from the full thickness of the specimen which does not allow most of it to be in focus to the observer. The confocal microscope eliminates this out-of-focus information by means of a confocal "pinhole" situated

in front of the image plane which acts as a spatial filter and allows only the in-focus portion of the light to be imaged. Light from above and below the plane of focus of the object is eliminated from the final image.

The microscope uses a special "beam splitter" (*"dichroic mirror"*). This mirror reflects light shorter than a certain wavelength, and passes light longer than that wavelength. Thus your eye only sees the emitted red light from the fluorescent dye, rather than seeing scattered purple light. The purple and red bars next to the dichroic mirror represent additional filters to help prevent the different wavelengths of light from going the wrong directions.

While the image that is seen with confocal filtering is all in-focus information, this creates another problem. Compared to a normal fluorescence microscope, the amount of light that is seen in the final image is greatly reduced by the pinhole, sometimes up to 90-95%. To compensate for this loss of light somewhat, two components have been incorporated into modern confocal microscopes. First, lasers are used as light sources instead of the conventional mercury arc lamps because they produce extremely bright light at very specific wavelengths for fluorochrome excitation. Second, highly sensitive photomultiplier-detectors (PMTs) were employed as imaging devices to pick up the reduced signal. The signal for detection in the original design of modern confocal microscopes is created by scanning a focused laser beam across a square or rectangular field. A system of motorized scanner mirrors sequentially scans a horizontal beam across the specimen.