# Optical Spectroscopy and Microscopy

**Prof. Balaji Jayaprakash**
**Centre for Neuroscience**
**Indian Institute of Science – Bangalore**

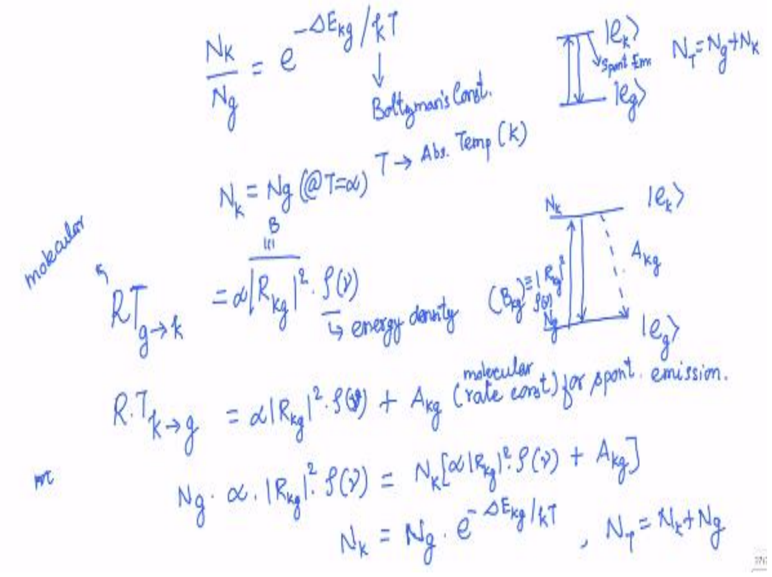
## Lecture – 15
## Fundamentals of Optical Measurement and Instrumentation

Hello and welcome to the lecture series on an optical spectroscopy and microscopy, and so far what we have seen is, we have talked about how we can express the rate of transitions involving energy eigenkets of an atomic or system of chromophore and how the that can be expressed in terms of the molecular properties itself and then predicted 2 important transitions, the absorption and the stimulated emission.

Following which I actually was taking you to a phenomenological approach of Einstein and said that how he predicted that you need a spontaneous emission process from the arguments of Maxwell-Boltzmann distribution and the thermal equilibrium. In this class, what we are going to see is that we are going to set up the equations that describe the rate of these processes and then with the idea of getting an expression for describing the spontaneous emission in terms of the molecular properties.

Perhaps in terms of the stimulated emission are the absorption coefficients so that we can actually estimate the spontaneous emission rate from the molecular properties okay. So here we go.

**(Refer Slide Time: 02:21)**



$$\frac{N_K}{N_g} = e^{-\Delta E_{kg}/kT}$$

k → Boltzman's Const.

T → Abs. Temp (K)

$N_T = N_g + N_K$

$N_k = N_g$ (@$T=\infty$)

molecular

$$RT_{g\to k} = \overbrace{\alpha|R_{kg}|^2}^{B} \cdot \rho(\nu)$$

↳ energy density

$(B_{kg}) \equiv |R_{kg}|^2 \rho(\nu)$

$$R.T_{k\to g} = \alpha|R_{kg}|^2 \cdot \rho(\nu) + A_{kg}$$

(molecular rate const) for spont. emission.

$$N_g \cdot \alpha \cdot |R_{kg}|^2 \cdot \rho(\nu) = N_K[\alpha|R_{kg}|^2 \cdot \rho(\nu) + A_{kg}]$$

$$N_k = N_g \cdot e^{-\Delta E_{kg}/kT}, \quad N_T = N_k + N_g$$

So let us look at our where we are in the previous class. We were looking at the energy level diagram and marked down what are all the different processes that can happen okay. The upward arrow representing the absorption and the downward arrow here right next to it represents the emission, but both these processes are happening in the presence of light okay. So this is a stimulated emission and apart from that we have a process that is represented by this cross arrow. We call that as the spontaneous emission.

Of course, this reaches to the ground state too, but I am just for differentiating that maybe I can actually represent it in a different way, which is like that, it is a spontaneous emission process and we know that the absorption rate or the rate constant is directly proportional to the molecular property Rkg square actually times the energy density. So here since it is in a thermal equilibrium, you can think of that as the rho as a function of the frequency or omega whichever you like to think of.

So now at equilibrium, the forward rates and the reverse rates are the same. So let us write down the transition rate for rate of transition for process going from g to k. So we have one process that is given by R, the modulus square Rkg times rho of nu, the energy density okay. This is energy density with which I mean since the whole system is in equilibrium with the black body right, so we need to know the energy density, I mean the energy density here is the perturbation, the radiating energy from the black body that is interacting with the system.

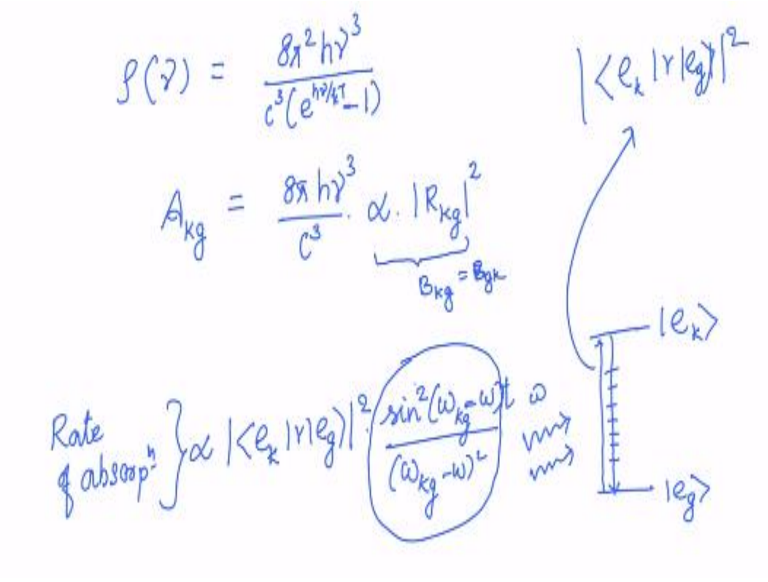
On the other hand, the rate of transition for all the processes going from k to g is given by Rkg square right. This is basically this whole term Einstein called it as B coefficient, actually there are some constants which I kind of ignored here, but we can put them there some alpha. So, we can think of that as alpha Rkg this thing and this alpha Rkg modulus square this is the term that we obtained from our Fermi’s golden relationship and Einstein called it as B coefficient plus the important term here which is Akg.

Now this is independent of the light radiation, so we just call it as Akg. What is Akg here? It is the rate constant okay, molecular rate constant for spontaneous transition, very good. So now we have written down that the molecular rates of this processes, but if you want to equate these 2, what is really equal is not the molecular rates but actually the rates of the total that the molar rates right. So rate of the reaction so to speak is equal, the forward rate is equal to the reverse rate.

That is given by all the transitions that are happening from the ground state to the excited states to be multiplied by this corresponding number densities which is Ng. So we are going from the molecular to the molar rates. These RTs remember are molecular rate of transitions. So for an ensample of molecules, then we have to multiply that by the absorbed rate. We have to look at the ensample of molecules that means that we need to multiply this by the appropriate occupancy or the number density of the molecules in its states.

So Ng times alpha times Rkg square, alright. Remember this whole thing Einstein called it as B and hence he has B coefficient times rho of nu okay, at equilibrium should be equivalent to Nk times alpha Rkg modular square times rho of nu. So this is a stimulated emission term plus Akg alright. Now we also know that Nk = Ng times e to the power -delta Ekg by kT from the Boltzmann's equation and N total is Nk + Ng, okay.

**(Refer Slide Time: 11:02)**



Now using this along with the fact the whole system is in equilibrium with the blackbody radiation and for the blackbody radiation, the energy density at a given frequency nu is given by 8 pie square h nu cube c cube, times e to the power h nu by kT - 1 alright. So we use all these 3 relationships in the above expression and then what we could do, what we will be able to see is that the Akg right, the spontaneous emission rate constant corresponding to that can be written as 8 pi h nu cube by c cube times alpha times Rkg square.

I am just going to confirm we are not missing any terms here, okay. So remember since it is dependent on the stimulated emission term, he called it as the B coefficient right, Einstein called it as that was his Bkg okay. It is equivalent to, we know that it is same as Bgk okay. What you can see from here is that for a given molecule once you have them in a state k that molecule is going to come to a lower energy state the ground state with the rate constant Akg. So let us say somehow we prepare we take the molecule to the excited state okay, how can we do that?

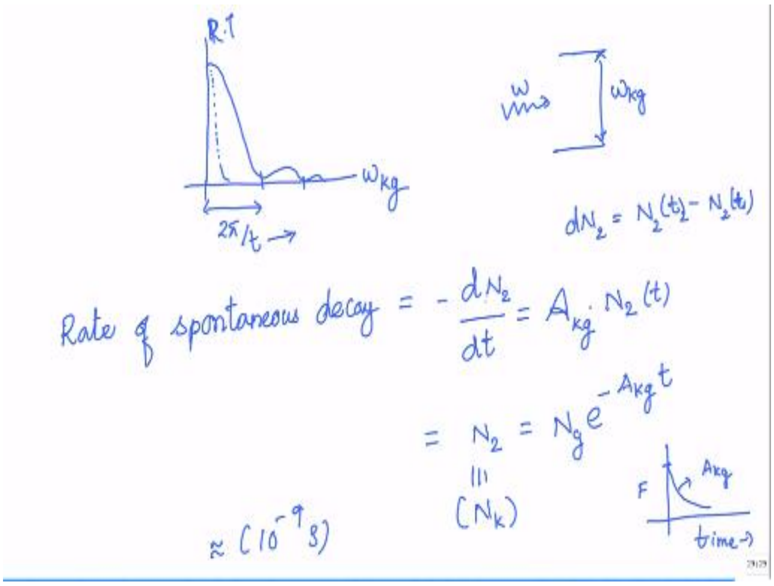
We have a system which is eg and ek. So now I am going to come in with my light radiation of some omega okay. Now if I shine this light omega, I know that 2 processes can happen, this as well as this. However my n2 or the nk is much lower than ng, I can practically neglect this process. So when I shine the light there is a probability that the light will interact with the system and then go to the ek state. Now this transition probability depends on few things. One is the nature of eg and ek, how?

Because its the relationship between the ek and eg that determines the value of this matrix element, right, and then we know that this rate is proportional to this matrix element so definitely that is determined by the ek and eg. We also know if you look at our expression for the transition probability ek to the first order that our omega has to be of a certain kind, omega needs to be close to omega kg, the transition rate as a function of omega can be thought of as from this expression.

So I am going to quickly go back to that expression and then write it down, so you can think of the rate of this forward absorption process okay. Now rate of absorption, first thing we said is directly proportional to rate of ek or eg square, and if you remember we had also this term sin square omega kg - omega term corresponding to the absorption. We completely ignore the emission process omega t divided by omega kg - omega.

So now if you plot this term, this tells you how does the transition probability at the rate of absorption change when you detune the system from omega kg, omega kg is the energy gap between the g and the k and now I am going to come in with the light of different frequency omega and now I can actually plot this.

**(Refer Slide Time: 18:08)**



$$dN_2 = N_2(t_2) - N_2(t_1)$$

$$\text{Rate of spontaneous decay} = -\frac{dN_2}{dt} = A_{kg} \cdot N_2(t)$$

$$= N_2 = N_g e^{-A_{kg} t}$$

$$\underset{(N_k)}{\parallel}$$

$$\approx (10^{-9}\,s)$$

When I plot this, you know it is sin function which is, this is my probability okay of transition or we can also think of this as rate of transition, now that is going to have a function like this where we are plotting omega. We are plotting this probability, the rate of transition as a function of omega kg. It was not a great picture, so let us see okay. What we have done is we have taken a light radiation of a fixed frequency omega and then we are going to ask as the energy gap between these 2 given by omega kg changes, how does the rate of transition change with respect to absorbing the light omega?

It is equivalent to saying that I am having a fixed omega kg and how does my probability of the light absorption for a different frequency omega look like okay. So now what you see is that, it has this particular function is called a sin function. So what we expect is that the transition rate is really high right and this is with respect to the interaction time right, t okay. So now if you look at the gap between this is given by 2 pi by t. Now you can go ahead to the previous expression solved for these zeros and you will be able to see that the gap is 2 pi by t.

Now if I make this t, the interaction time okay very long, I mean long compared to 1 over t that is comparable to that of, we are neglecting this omega kg - omega right that is how we get that. So when we do that, then what it practically amounts to is to say that the transition falls down really sharply okay. So you need to have the energy matched with that of, that is omega kg need to be up close to that of the omega and as you deviate from there, it is going to fall down towards 0 in this nonlinear fashion okay.

That is what has been observed in the Rkg and that is what has been coming in when we are trying to actually estimate Akg which is the spontaneous rate constant for the spontaneous transition. So apart from the light taking the molecule from the ground state to the excited state, by the virtue of the molecule going from the ground state to the excited state, now it is going to come back to the ground state okay at this rate by emitting and at the rate given by Akg times the number of molecules in the excited state.

Now when this happens through an emission of light radiation, we call it as fluorescence. Again once the molecule goes to the excited state, the radiative decay of this molecule from the excited state to the ground state by emitting a light photon through a spontaneous process we call that as fluorescence. Again now if this whole process were to be true, we should be able to measure the rate of fluorescence decay and thereby be able to obtain a number for Akg or at the least the fluorescence decay that we observe experimentally should correspond to the functional form of the numbered decay of the excited state ek okay.

There are molecules in the excited state k and their number is going to decay, I mean that number is going to change as a function of time, it is going to come down as a function of time. We know it is decaying I mean because it is going to come down as a function of time and this function can be obtained analytically. Since we know this is a rate process, we can go ahead and then solve for this expression, rate of transition from k to g setting rho v = 0. So in which case the spontaneous decay rate are in the absence of the light radiation, I mean instant light.

Now here I can safely call it as rate of spontaneous decay is given by -dN2 two by dT, sorry it is rate of decay, we do not have to write, where dN2 is given by N2 at time t2 - n2 at time t1. We know that t1 is going to be larger compared to t2 because it is a decay process, that is why I explicitly put that minus here as -dN2 by dt. I can indicate that as this = Einstein's A coefficient corresponding to Akg times N2 itself. Now, we can go ahead and solve for N2, now N2 will be N2 as a function of time and we will see that this N2 will have a form.

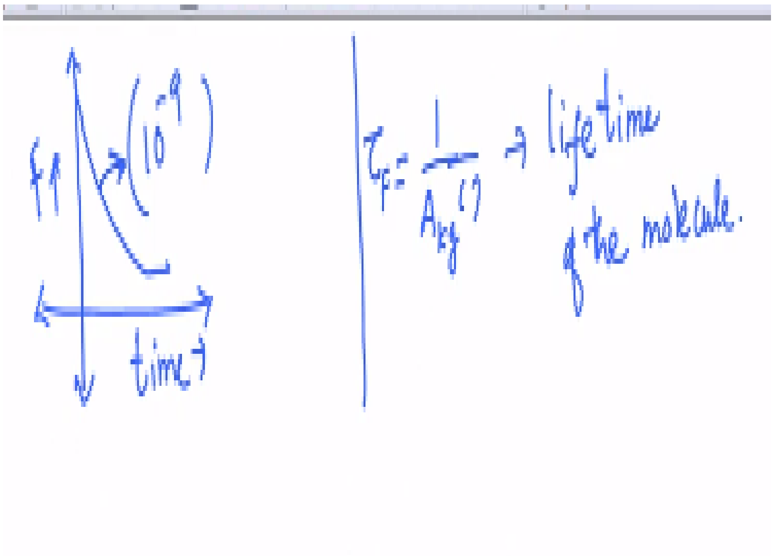
We have been calling k and g, so I am going to replace that 2 as N2 = Ng times e to the power -Akg t. Now N2 to be consistent with our previous descriptions is nothing but our Nk, number of molecules in the kth state okay, so that is the number of molecules in the excited state okay. So what we should see is that this N2 should be very high initially right after you excite okay and after you turn on the light right. You have made the molecules to go to the excited state and then immediately you turn off and then like with huge speed, I mean very high resolution, you are counting the number of molecules in the excited state.

Now that number is going to start with some value here and then going to come down at a rate given by Akg okay. Now this whole process, actually we can go ahead and calculate given the molar extinction because the rate constant Akg is related to the molar extinction coefficient and the Rkg and alpha and so forth because Bkg and Rkg are related. We can go ahead and write down those expressions, I mean put in those numbers and you will see that will come to be of the order of nanoseconds, it is 10 to the power -9 seconds.

Now this is a very fast process. To monitor this number okay with this high, so this is you can see the fluorescence per se basically is reflective, the number of molecules that you are actually seeing in the excited state proportional to that the number of the decay is, right. The rate of the decay dN2 by dT is proportional to the number of molecules N2 or Nk here at any given time t. So if that number is high, you are going to have a higher rate of transitions.

As a result, there will be higher rate of emission which means higher number of fluorescent photons that are going to come out. So now if you plot that as a function of time, this is the form that you get, an exponential decay form.

**(Refer Slide Time: 30:35)**



So to measure this exponential, as I was telling you that as a function of time, though to measure this exponential is a pretty tedious process because the timescales of this is in the nanosecond range okay. So we need special detectors electronics and methodology to be able to see changes occurring at this timescales. Later in the course, we will talk about methods using fluorescence that we use to measure these rate processes and the time constant corresponding to this rate constant process.

We can think of that as tau F is given by 1 over Akg here and any other constants that I have missed. So now this is called as the lifetime of the molecule. This lifetime again since Akg is related to the molecular property is a molecular property and it will serve as a fantastic diagnostic tool to tell you about quite a few things starting from the environment of the molecule, what happens to the excited state, so on and so forth.

We will see how we can utilize these processes, this fluorescence, to follow and study either the fluorescent molecules themselves directly or we can use the fluorescent molecules as a tag to study some of the systems later in the course, but for now I want to say lifetime called the tau F defined as 1 over the rate constant of the spontaneous process is essentially the time the molecule spends in the excited state before it comes down all by itself without the presence of light okay.

Now in the next class, we will see how we can utilize our understanding of these transitions and the quantum mechanics to further our description of the system and then say we really do not have to plug in a process just the way Einstein has done. Einstein said there has to be, he reasoned out there has to be a spontaneous emission right, it does not come naturally from the basic principles. So now what we can do is if you actually treat the light itself in a quantized manner, we will see quickly that it comes out very naturally.

The spontaneous emission comes out very naturally as a part of this whole process just the way stimulated emission came out naturally while we were trying to describe the interaction of the light and the matter to understand how the absorption process happened. I will see you in the next class. Thank you.