Basics of Fluorescence Spectroscopy
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Lecture – 21

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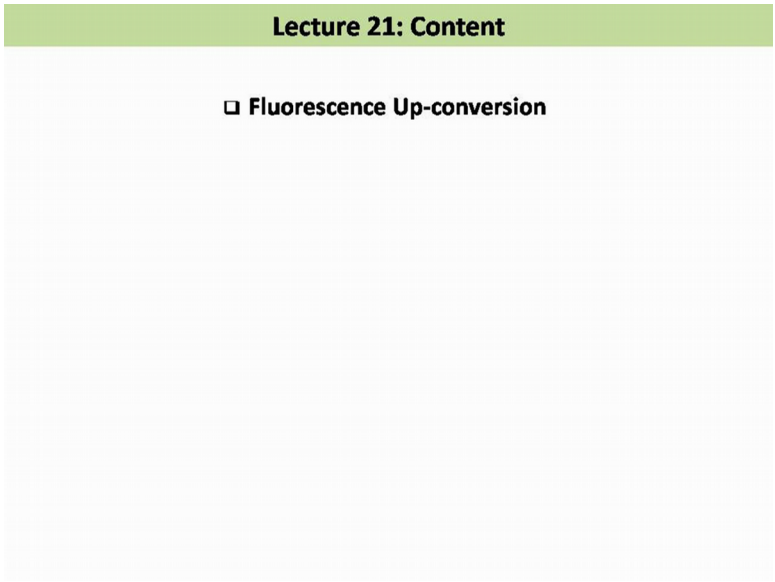

Welcome to the lecture number 21. Till last class what we are discussing is the time correlated single photon counting set up. And I already mentioned you that we will going to discuss two types of method for the life time measurement.

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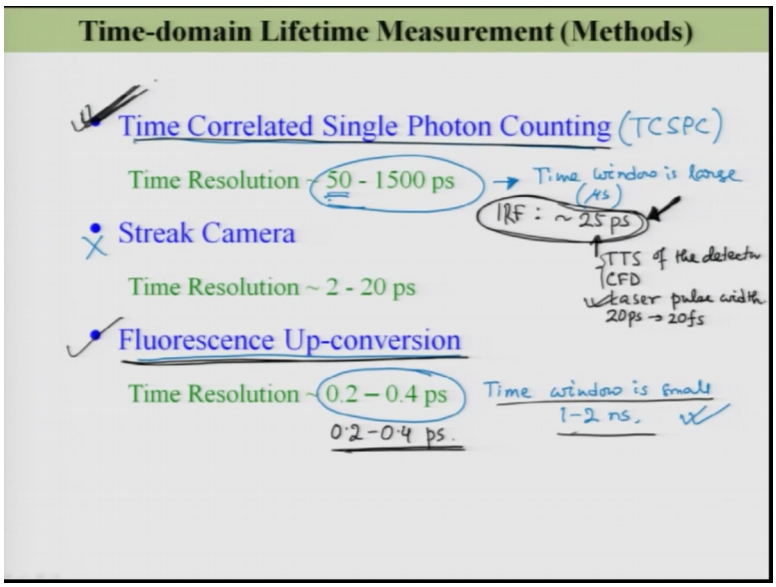

So, the first one which I have already discussed is this time correlated single photon counting. And probably by now you have idea about the principle of the system and how does it work. Now, we will going to discuss the fluorescence up conversion.

Let me tell you that each of these method has their it is own advantage as well as disadvantage. Let me talk about the disadvantage: for this time correlated single photon counting or this TCSPC. The disadvantage is the IRF even for the very modern set up the IRF is just above 25 picosecond. It means that if you want to measure the dynamics of any such kind of process involving the fluorescence, the dynamics you cannot measure it dynamics which is less than the 25 picosecond. But actually because of these reiterative reconvolution technique one can report a time constant of one-tenth theoretically; one-tenth of this IRF. So, even that it could be only 2.5 picosecond or 3 picosecond.

So, if something is happening less than that time scale, you will not going to have any clue about the time scale of that process. So, you need a better time resolution setup; that means, IRF must be in that time resolution. That means, above 0.1 or 0.2 picosecond then you will be able to find out those types of dynamics. So, for that this fluorescence up conversion is the technique where the time resolution is about 0.2 to 0.4 picosecond; as for this region over here. The disadvantage here: the time window is small, it could be 1 to 2 nanosecond, but here the time window is really large it can go to the micro second or milli second time region.

Yes, I already have discussed why these IRF is limited to 25 picosecond; the reason behind it is the transit time spread of the detector. Another reason is that the inherent that CFD; what is that form of the CFD. And the third one is the laser pulse width. Even I change this laser pulse width from 20 picosecond to 25 picosecond I have already showed you that there will be no change in the IRF, because this IRF is mostly guided by these two.

And you cannot buy a better detector than this what detector is has been used to get this 25 picosecond IRF, we cannot buy that better detector than this. It means that if I follow the working principle of this TCSPC system I will never be able to achieve a time resolution or IRF less than 25 picosecond, but if you are interested in measuring the dynamics which is much faster than these then what you have to do; you have to go for a different type of arrangement by which you will be able to get a much much shorter IRF.

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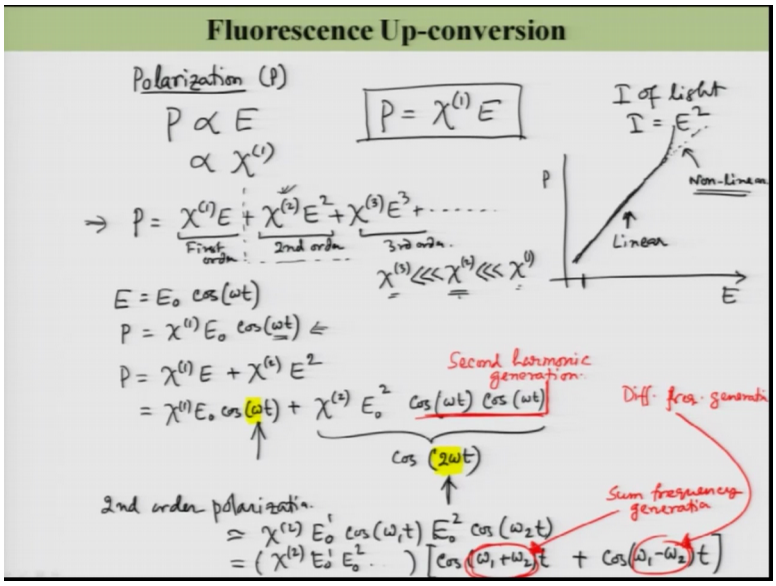

So, that technique is the fluorescence up conversion technique. And to discuss this technique I need to discuss a few things, just too few things otherwise I will not be able to discuss this technique. So, that thing is the polarisation, usually denoted as P. What is polarization? So, when a substance or material is placed under the electric field then there is a induce dipole moment because of the oxidation of this electric filed right inside this material. So, induce dipole moment per unit volume is known as the polarization.

So, this polarization is a function of electric field that I know, and for some molecule it is easy to polarize it and for some molecule it is not easy to polarize the same. That means, for some molecule the creation of induce dipole moment is more and for some substances the creation of induce dipole moment is less. So, this is the molecule property. So, that is also proportional to molecular property chi 1.

So, I can simply write this P is equal to chi 1 E. So that you have probably seen this type of equation, this is the polarization. So, if I increase the strength of this electric field; that means intensity; intensity is E square. Now, if I increase the intensity of the light then the induce polarization will increase, because E is increased so the induced dipole moment will increase; that means, polarization will increase of the system. If I now plot this P parses E then I will get a straight line, because this P is some constant into E. But if you extend it what people have seen that this line deviates from the linearity, this line

deviates from the linearity. And some non-linear effect comes into the picture. In this case this is linear over here it is non-linear.

So, this nonlinearity or the polarization was explained by this equation P is equal to chi 1 E plus chi 2 E square plus chi 3 E cube and so on. So let me take a functional form of these electric field; its cos omega t I can think of it right. So, with a amplitude E 0. So, lest say the E in this case; so this is the first order, this is the first order, this is the second order, this is the third order, and so on and so forth.

So, now if I think that E is equal to E 0 cos omega t, so this is the frequency. Then if only first order term is at a level right, so if I approximate that this equation is only till over here then what I will do I will simply write P is equal to chi 1 E 0 cosine omega t. So, you see that polarization that is induce dipole moment this polarization is also oscillates with this same frequency. That means, the colour of light which will come out of from here will be the same colour of this, because this is the same frequency the colour is the same.

Now if I take that P equal to chi 1 E plus chi 2 E square both the term, if you want you can take the third one or fourth one or so on, but please remember that is chi 3 is much much smaller than chi 2 and which is much much smaller than chi 1. It means that when the value of E is small yes this is much much smaller than these and this is much much smaller than these then the contribution will only come from the chi 1 E. The contribution from the second term, third term, fourth term will be negligible. The contribution from the second term will be there measurable only when the E is very high. Then E square will be very high and then although this chi 2 is much much smaller than chi 1 probably will be able to see some contribution.

And this kind of effect is only visible when the power of the light is very high which is the case for the laser, otherwise for normal lights such kind of non-linear effect is not visible. What with the laser? As I mentioned briefly the one of the property of the laser is that the laser has a very high power then only will be able to see this non-linear effect.

Now, let us take the same functional form as that what I have wrote over here in this case. So, I will just simply write here, chi 1 E 0 cosine omega t plus chi 2- here it will be E 0 square and this is cosine omega t and again cosine omega t the two times. So, this is

like a cos a cos b type of formula. So, this part will going to give you cosine twice omega t term.

So, what you see? You have these omega terms as well as this 2 omega terms, both the term is present for this polarization. That means, the light which will come out of the system will have both the frequency: the frequency that is actually heating the substance and just the double of that frequency which is 2 omega- please note here. So, you have this omega here and you have this 2 omega here. That means, the colour of light will change the frequency will get will become double.

Now, consider that you are using two different lights of frequency: omega 1 and omega 2. In this case that second order polarization you will be equal to chi 2 E 0 1; that is that one kind of light cosine omega 1 t and E 0 2 cosine omega 2 t this is a two different colour of this light at two different lights; that means this is chi 2 E 0 1 E 0 2. And then what I will going to have is although things will be there like let me take it outside. So, basically what I will going to have, is cosine omega 1 plus omega 2 t and I will also get another term plus cosine omega 1 minus omega 2 t. I will get these two terms.

So, you note here that this term is summation of these two frequencies and this is called the sum frequency generation. And in this case this is just a difference so this is known as difference frequency generation. What we have seen is that this is the sum frequency generation, and these case is a special case of this sum frequency generation when both the colour omega 1 and omega 2 they are same and equal to mega.

So, here this is the special case and this special case is known as second harmonic generation. That is it, with these background let me continue.

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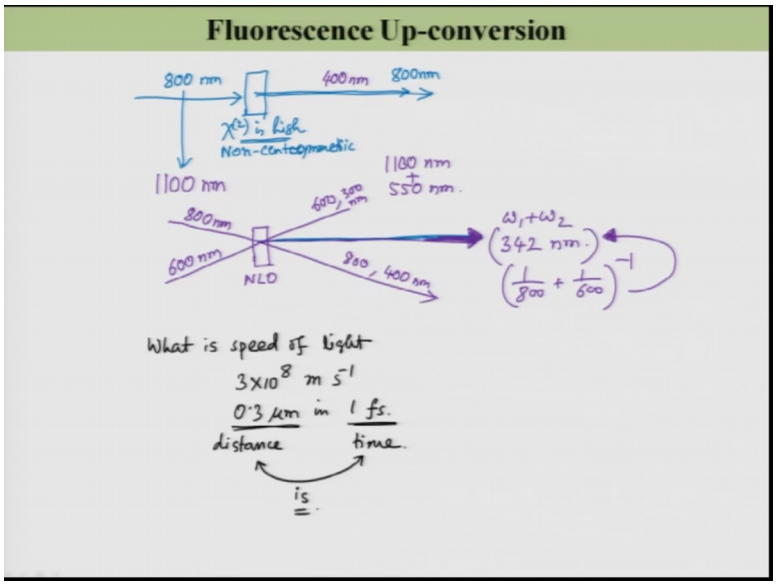

Let me also tell you that what is a implication of these. This means that if you have a light of 800 nanometre coming and hitting substance which is called the non-linear attic material. In that case the value of chi 2 is high compared to the other substance. And it has the special characteristics that it is a non-centrosymmetry. I am not going to discuss all the details because obviously the nature of this course. So, this has to be non-centrosymmetric in nature.

Then, that same 800 nanometre light will come through, but along with that another light that is if this is my omega then 2 omega light will also come. So, for this 2 omega it will be obviously will going to have a different colour. So, from here that 2 omega light will also come which is nothing but the 400 nanometre. Simply if you take not 800 if you take some other light let us say 1100 nanometre, then your output will be 1100 nanometre as well as 550 nanometre.

Now if I do something else, if I take the light one light along this direction and another light along this direction, and the colour of these two lights are not same. Let us say one light is 800 nanometre and another light is 600 nanometre. Then this 600 nanometre light after passing through this inner low material will produce 600 as well as 300 nanometre. And this 800 nanometre light will produce 400 as well as 800 nanometre light. But, these two light will also mix together NLO crystal and that will going to produce another kind of light which will come along these direction and that light will be the sum frequency

generation light. Here is my omega 1 plus omega 2. So, in this is case this value will be 342 nanometre.

So, this is the property so I am not going to describe exact all the mathematics behind it, but take it granted that this light will be along this path, and here this is simple right this is 1 of over 800 plus 1 over 600 inverse is going to give you this value. That is for sure, there is no problem. So, this is the just omega 1 plus omega 2, because this is 1 over I have just use this as a wave length. So, I change it to the wave number and then again inverse it. So, that is it.

Now, remember that such process will takes place only when both the lights are present inside the NLO crystal if only one light is present; that means, if only the 800 nanometre light will present then the output will be 800 and 400, if only 600 is present then the output will be 600 and 300, but if both 800 and 600 will present then only that some frequency will takes place and you will get a output along these direction with wave length of 342 nanometre. So, this is clear.

Now, another important information I want to give you then that will help me to tell you what is the fluorescence up conversion method. That information is what is speed of light? So, you all know the speed of light its 3 into 10 to the power 8 meter per second it means light travels a distance of 0.3 micron in 1 femtosecond. So, 0.3 micro meter in 1 femtosecond. So, this distance travels by this light. So, here you see this is my distance and here is my time. That means, these two are correlated with each other.

So, if I measure the distance I can estimate the time and vice versa. If you know the time then you can measure the distance. In this particular case, in this particular fluorescence up conversion set up I going to show you that in this case distance is my time.

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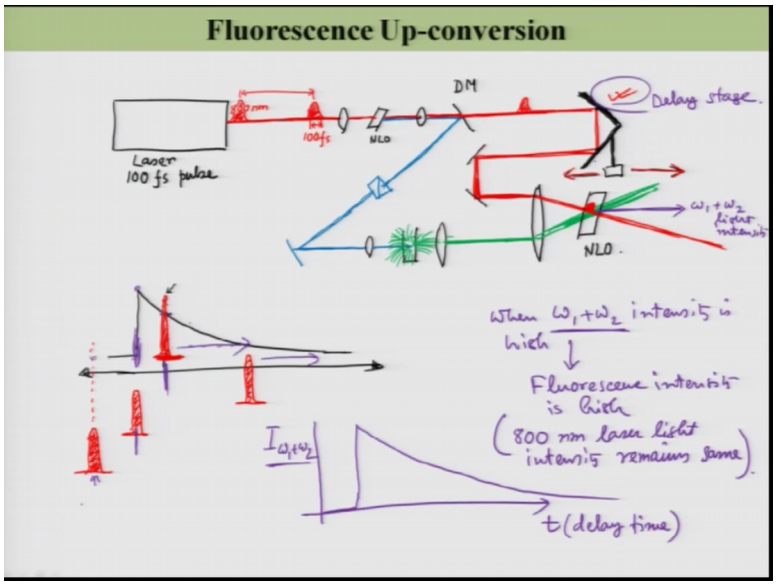

Now, let us proceed. Now let me draw a set up here. This is my laser which can generate very short light pulse, because now my aim is to have the instrument response function of in the order of 100 or 200 or 300 femtosecond. So obviously, if you use a light pulse of 200 picosecond then it is going to shows some function cannot be like a 100 or 200 femtoseconds. So, you have to use at least the light source as a 100 femtosecond light source. So, let me take this as 100 femtosecond pulse it can generate.

So now, this light what I will do, I will put some lens and this in a loop system and another lens until I put a beam splitter over here. So, this is my NLO. Now let me take this red colour, because let us consider that the wave length of this emitted light from this laser wave length of this laser light is 800 nanometre let us consider; that means, the colour is reddish. So, it is giving me 800 nanometre light, but this light is not c w light- not continues wave this is the pulse light. So, light is present for some time and then rest of the time light is not present. Again probably at this position light is present. So, the difference between these two light is guided by the repetition rate of the laser and it has some width which is already I have said is 100 femtosecond, clear.

So, this is the laser light. So, the intensity is high, electric field strength is high; that means, it will undergo NLO, because I said when the light intensity is high then only it will undergo the NLO effect otherwise not. So, in this case when this red light will pass through this NLO that 400 nanometre light will also produce, because it is 800

nanometre, so 400 nanometre light will also produce. So, let me mark it like this blue. So, both the lights are coming from this NLO. And this beam splitter is a device where one colour of or this is not a beam splitter sorry this is a dichroic mirror. So, this is a dichroic mirror, this is the device where this one colour of light will pass and another colour will reflect. For example, in this case the blue light is getting reflected and this red light is transmitted through this dichroic mirror. So, I will get this red light at this side and the blue light at this side.

Then we will do couple of thing over here which will put a device red which I will going to discuss later. This device is the same device which I have skipped during the discussion of the TCSPC system. I said the polarizer again here is similar kind of thing which I will going to discuss it is a horizon, but necessity of these I will going to discuss later.

Then this light will be used let just you can put another mirror over here and then this light can be used to through some lens to excite your sample, here you have lens and here you have your sample. So, you can excite the sample with this blue light. And then sample will generate the fluorescence. Fluorescence is typically the colour of fluorescence is different; let us I choose this green light as a fluorescence. So, fluorescence will come out in all the directions. So, this is my fluorescence light is coming out in the all the direction. And you put something over here some mirror or something or a big lens to collect whatever the amount of fluorescence you can collect.

Then this fluorescence will be collected in this position. So, as much as you can you collect the fluorescence and this fluorescence will be guided along this pathway. What you do with this red light? You take this red light and make it come back from here to here to here and put a mirror later and here and you make it come over here. But in this case what you will do, you use a mirror system which is moveable. Just show you here; you use a mirror system which is moveable.

This is a mirror system; that means, when the light will fall here it will be reflected from this to this and then it will go. But this whole system is mounted on a stage which you can either push in this side or you can take it in the front direction. And then you have a mirror here, you have another mirror here, and you put a big lens over here so that this light will going to focused in this pathway and your fluorescence will also be focused

along this pathway. And at these junction where both this radiant the fluorescence actually overlapping what you do you put 1 NLO crystal, over here. That is it, this is my diagram.

Here what I am trying to do. Now, this fluorescence will have its decay; it has a decay it is a time dependent fluorescence. But these pulse is remain as this 100 femtosecond pulse because some of the pulse has converted only the intensity will go little down, but eventually this pulse will be steel here over here. So, these pulse will come over here. When this pulse will come over here, that you can guide by pushing these delay stage either in this direction or in this direction, that when you want the pulse to be come to come here.

Now let me draw this fluorescence intensity decay of the sample and this pulse in this diagram below. Suppose this is your fluorescence intensity decay, and this is the position. Now this fluorescence intensity decay will be there, it will be there for a given excitation pulse the fluorescence will come at initially the intensity is maximum then as time goes the intensity will become smaller and smaller and to very long time this will going to be 0.

Now, when these femtosecond pulse 800 nanometre femtosecond pulse will come that you can guide by moving this mirror system. So, you can choose that 800 nanometre pulse will come at this point of time then the total intensity will be proportional to the intensity of this 800 nanometre light, and the fluorescence intensity over here. So these is moveable, you can actually put wherever you want these 800 nanometre that red one let me draw it in red colour then it will be easy for you to understand. So, this one you can actually move great vertically. So, if this is coming later at this position that means it will onsite over here. So, at your desire it can be here, it can be over here, it can be over here, it can be over here. So, depending on the position of these two mirror system.

Now, consider the case when these positions of this femtosecond pulse that 800 nanometre pulse is at this position where there is no fluorescence intensity. What will happen in this case? In this case these fluorescence intensity as 0; that means, one of the component is 0. You remember that omega 1 and omega 2 omega 1 plus omega 2, but it has its E 0 1 and E 0 2 for these two components, if one of them is 0 that product will be

0 automatically. So, I will not going to get any signal in these direction; I will not get any signal in this direction.

But, when the system is at this position is just matching around these positions. So, the intensity will increase little bit. But when the system will be at this position then there is a lot of intensity of the fluorescence and this remains fixed, this 800 nanometre light that one remains fixed. So, the intensity will increase. And as you move it from here to here the intensity will again decrease, and if you move it from here to here the intensity will be much much smaller. That means, by scanning the position of this 800 nanometre pulse throughout this fluorescence decay what I can do, I can measure this omega 1 plus omega 2 light intensity when omega 1 plus omega 2 intensity is high I can simply say the fluorescence intensity is high provided that 800 nanometre laser light intensity remains same there is no fluctuation on that.

So, I can plot the intensity of omega 1 plus omega 2 light as a function of the displacement here. And you remember I said that light travels 0.5 micro 1 to second. So, if I plot displacement you can readily convert that displacement with time. So, displacement is time for me in this case. So, I can plot the time. What will happen? When this light for 800 nanometre light is at this position then I will get 0 intensity over here, when it is over here the intensity will increase little bit, when it is over here then intensity will increase maximum, then the intensity will decay, decay, decay, decay, like that.

The intensity profile of this omega 1 plus omega 2 as a function of t this is my delay time will be exactly same as the original fluorescence decay; original fluorescence decay provided the 800 nanometre light intensity remains same during your experiment, that is it. So, in this case what you will going to see is that the time measurement is being done by the distance there is no electronics is involved for the time measurement. In TCSPC the electronic part the detector was its itself was involved the for the time measurement. The CFD was used to reduce that time retour induced by the amplitude retour of the PMT. So, those electronics parts, and the tag was there that was working as a stopwatch, but here the time measurement is being done by these delay stage.

And you go to any mechanical engineer or any shop that can create a mechanical device with a micro meter screw by which you can translate this 2 meter system with the

precision of a micron. If you can move this as a precision of a micron then right then like you have done you can measure the time in that 1 femtosecond time region. So, if you can measure the time in 1 femtosecond time region it should be able to get very high time resolution. That means, very very plot IRF. And in this case the IRF only depends on the pulse width of the laser. There are some other factors like group velocity expression these are where I have decided not to discuss all those things.

So, if you start with 100 femtoseconds light pulse laser light then your IRF could be 150 second, those small changes because of mainly because of the GVDM and other case other thing which I have not going to discus.

So, in this case what you can see is that ASD set up is only optical and the time measurement is being done by the delay stage just by that changing the distance that is the measurement of the time. So, in this case the IRF will be very similar to the laser pulse. So, in this case the IRF could be 150 femtosecond or 200 femtosecond.

And this IRF can easily measure by the Raman scattering in this case which if some of you are interested then write me and I will be happy to explain. So, that is all for this fluorescence up conversion. So, we have discussed two methods basically the TCSPC and fluorescence up conversion. And here we will finish our this like to measurement think, and we will continue at a discussion on other topics from the next class.

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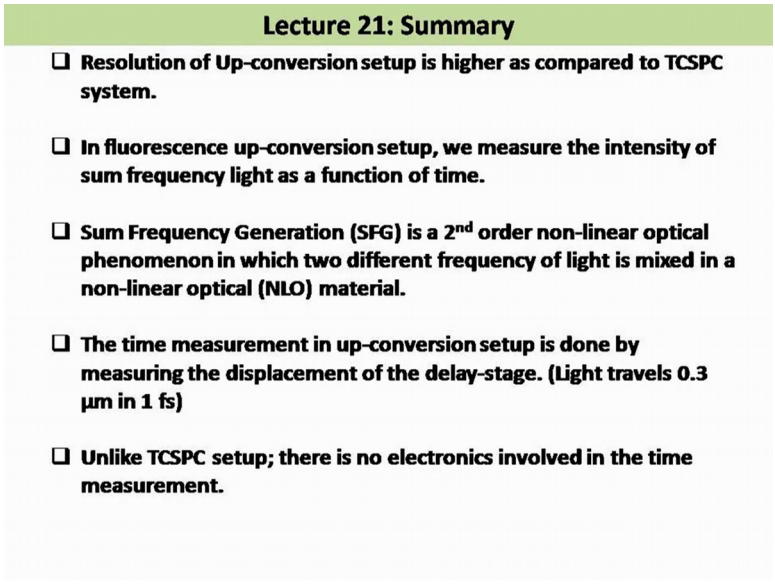

Thank you very much.