Basics of Fluorescence Spectroscopy
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Lecture – 18

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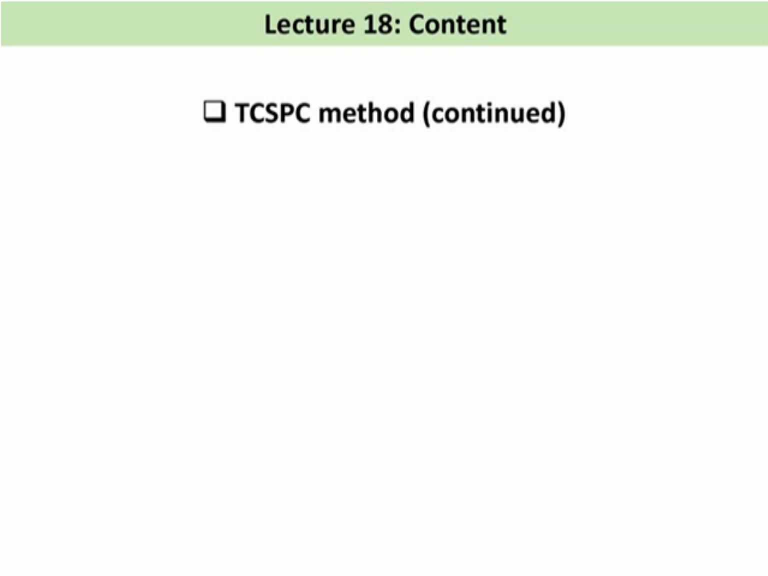

Welcome to the 18th lecture of these course Basics of Fluorescence Spectroscopy.

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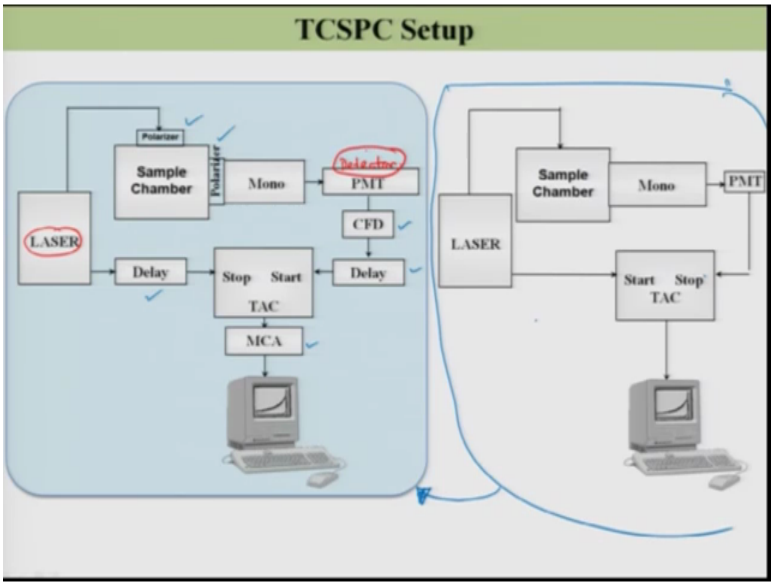

On the last class what you are discussing is TCSPC setup and what I showed you is this setup which is over here the right side this setup where which I discussed. However, if you look at the exact the actual TCSPC setup, that actual TCSPC setup has more component than what I discussed yesterday. So, you have here the CFD, you have this delay over here, you have this polarizer over here and you have the MCA, but I have not discussed all those things. So, to discuss the necessity of the CFD or delay or this polarizer we need to discuss little more about the different component of this basic TCSPC setup.

So, let us start discussing from the laser part. So, what is laser? Laser is abbreviation of the light amplification by the stimulated emission of radiation. So, for us right for us means that for this TCSPC set up.

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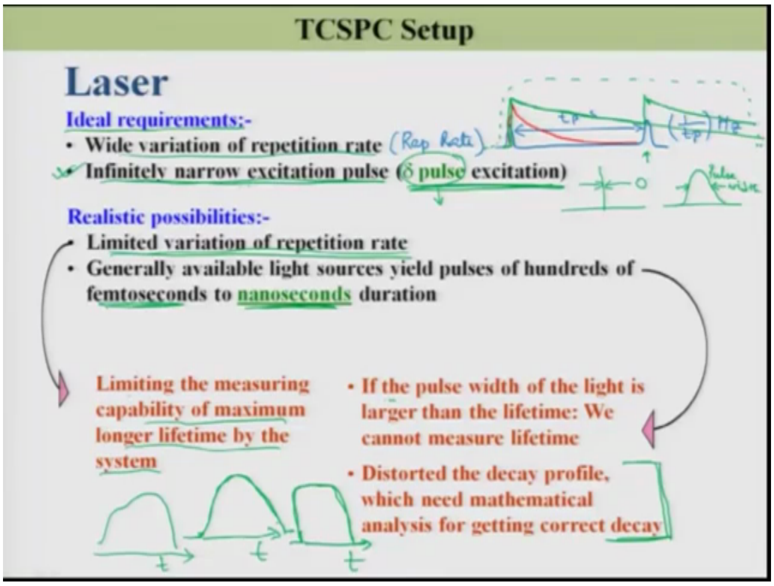

What we need? We need wide variation of the repetition rate. So, here this is my rep rate sometimes is also called that means, the time delay between the two successive pulse should be varied as per our desire. So, if these are the two successive pulse, if the time with time delay between these 2 pulse is t p right second then the repetition rate is 1 over t p hertz.

So, that repetition rate should be varied as per our desire. When I need the more the less repetition rate or more time interval between the 2 pulse when the fluorescence decay is much longer. Suppose the fluorescence decay is originating from here and ended here, then t p time delay between the two successive pulse is fine. However, consider this fluorescence decay is longer than these is the originating from here, it has to originate when the pump pulse will come and then it is going like this way is slower one what will happen if the repetition rate is again 1 over t p hertz, then while it is decaying another pulse will come. So, it will again excite the molecule to the excited state and then it will start decaying. So, the decay pattern will be very much distorted because of the presence of this pulse.

Now, in this case what I need? I need the repetition rate to be much smaller so that the time delay between these two successive pulse are much longer which will be beyond the 5 tau of this fluorescence lifetime. So, another requirement is infinitely narrow excitation pulse that is what I said yesterday right. So, that is what we are discussing this delta pulse excitation; delta pulse means the light is present for some time where the width of this line is 0. However, our in real life in such kind of pulses is not exist.

So, we have some time width of these spectra. So, it is like this. So, I have some pulse width over here. Now question is if this pulse width is very high right if it pulse width is more than this lifetime, let us say the pulse width is something like this. So, light is on for this time; obviously, I will not be able to measure this decay. So, I need the as much as short pulse as possible for this kind of TCSPC system. So, these are my requirement from the point of view of laser, I will show you the point of view of all other different components like CFT, like PMT and all other things, but for the point of view of laser what my ideal requirement. Ideal requirements are wide variation of the repetition rate so that I can tune it and the infinitely narrow excitation pulse that is the delta pulse excitation.

But in reality right we always encountered a limited variation of the reputation rate. So, for a particular laser the reputation rate is say for example, one megahertz if even you wish to change the repetition rate to one kilo hertz it is not possible. So, we are restricted about that, and also the pulse width cannot be generated as a delta pulse and it depends on the laser medium, sometime it is nanosecond duration, sometime it is 100femtoseconds duration and so on and so forth we also have the picoseconds laser pulses.

So, as you understand that this limited variation of the repetition rate will limit the measuring capability of the maximum longer fluorescence lifetimes of the system right and for this certain time width of this excitation pulse, I will limit our measurements of the lifetime if the lifetime is shorter than the pulse width then it will be difficult for us to measure that lifetime. So, for us the pulse width should be shorter than the fluorescence lifetime of the sample.

And also this another important part is here I have written which tells you that when you have a excitation pulse which is not delta pulse; that means, it has some shape it could be a Gaussian shape the shape could be like this shape, could be like these shape could be like these, like what about the shape but these particulars shape of the excitation pulse this is in the time axis right. So, please note that these are in the time. So, light is off here is on and then again it is off here light is off here again is turned on and it straight for some time the shape is like this and then light is again off right so like that.

So, that is eventually going to distort the decay profile. So, in that case I have to do something to get back the original decay right that is called the Iterative Deconvolution process that are going to discuss let us continue with this laser.

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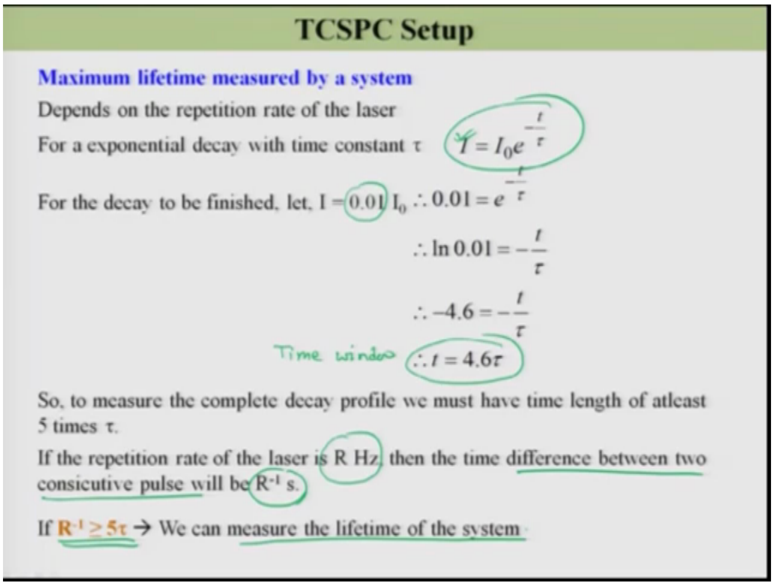

So, as I said earlier that if your system will undergo a single exponential decay, then for this exponential nature of the decay the I will be equal to 0 only when that t equal to infinity, but let us consider that I equal to 0.01 that is just 1 percent of I 0, that I believe that this is going to be 0 intensity, then you can simply calculate t equal to 4.6 into tau; that means, roughly 5 times of the tau if you allow this time window.

So, this t right here this t is my time window require to measure the decay. So, if I have these 5 times of the tau time window, then I will be able to measure the fluorescence decay otherwise not. So, that is the part of the repetition rate. So, if the laser repetition rate is r hertz then the difference between two between two consecutive pulses will be r inverse, second and when r inverse is greater than 5 tau then will be able to measure the lifetime of your sample by this TCSPC system.

Now, let me show that basic principle of this laser because when I will discuss fluorescence up conversion this part will be required.

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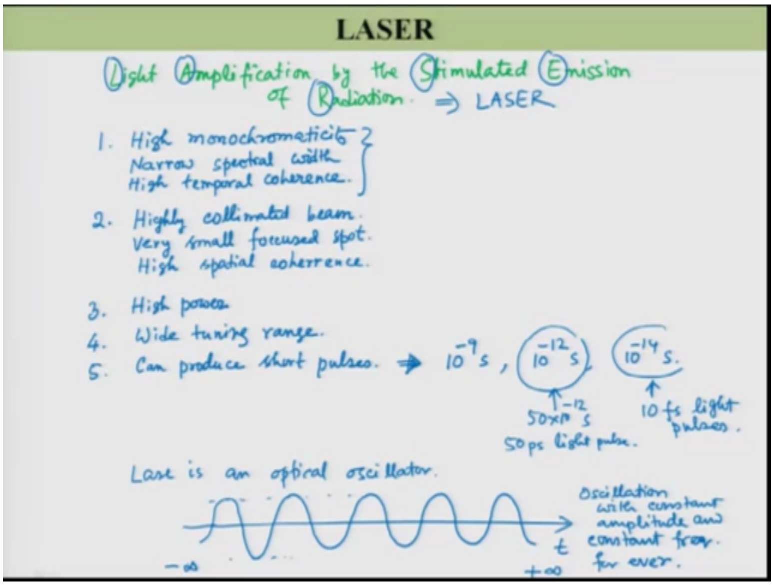

So, as I said that laser is light amplification by the stimulated emission of radiation right. So, I have this L A S E R that is what it is called laser. So, laser light right has some unique properties compared to the conventional light source; conventional light source means your tungsten lamp, your original lamp, your normal fluorescent tube light, CFL, LED bulb right those are the kinds of a light source, but laser light has some unique properties compared to such kinds of conventional light source.

What are the properties let us see; property number one lasers are generally high monochromaticits. So, laser lights are monochromatic light, I can also write that same property as narrow spectral width there is the same thing right? I can also say write the same property as high temporal coherence right these are all the same thing right these are all the same thing it tells me that lasers are monochromatic.

The second property of this laser is it can produce highly collimated beam right. So, it means that the lights which are coming from the laser they will not going to diverge come similarly to the normal light source conventional light source. I can write the same property as very small focused with spot it can produce, the same property can also be written as high spatial coherence.

The third property of the laser is it can produce high power. So, the powers of these lasers are much much more much much more than the conventional light source. Fourth property is laser sometime can have wide tuning range and the fifth property which actually we are interested for this particular discussion is that these lasers can produce short pulses depending. So we will going to discuss briefly about this fifth property because other properties will not going to discuss here. So, depending on the laser right we have different type of laser we have obviously, we have heard of that would be laser this a we have probably heard of the helium un laser and so on and so forth.

So, depending on the laser that means, the active medium of the laser it can produce a different type of pulse width. So, the pulse width can start from 10 to the power minus 9 second for some lasers, it can be 10 to the power minus 12 second for some lasers it could be also 10 to the power minus 14 second for some lasers.

So, as you can see when you will going to measure at the fluorescence lifetime which where the lifetime is about a nanosecond, then what kind of laser you need? You need such kind of laser which can produce at least picoseconds light pulses right if not picoseconds let us say 50 into 10 to the power minus 12 second, the 50 picosecond 100 picosecond something like that. If you are interested in much shorter time scale measurement then you have to go for such kind of light pulses. So, these are the 10 femtosecond light pulses, 10 femtosecond light pulses, here it is 50 picosecond light pulse.

Now, question is that from where the laser got all these properties. So, in one word I can tell that one of the important property of the laser is that laser is an optical oscillator. So, laser is an optical oscillator. That means that this electric field of laser will keep on oscillate with constant amplitude oscillation with constant amplitude and constant frequency forever. So, here my amplitude is constant and here is my time axis. So, it will keep on oscillator; if I go to plus infinity in this side is minus infinity in that side then if you keep on oscillator.

So, now if I take the Fourier transform of this right what you will get? I will get in the frequency domain this is in the time domain, I will get in the frequency domain and in the frequency domain you will get a sharp line right; that means, is just only one value of frequency in this case; so in this case that plus infinity, minus infinity oscillation.

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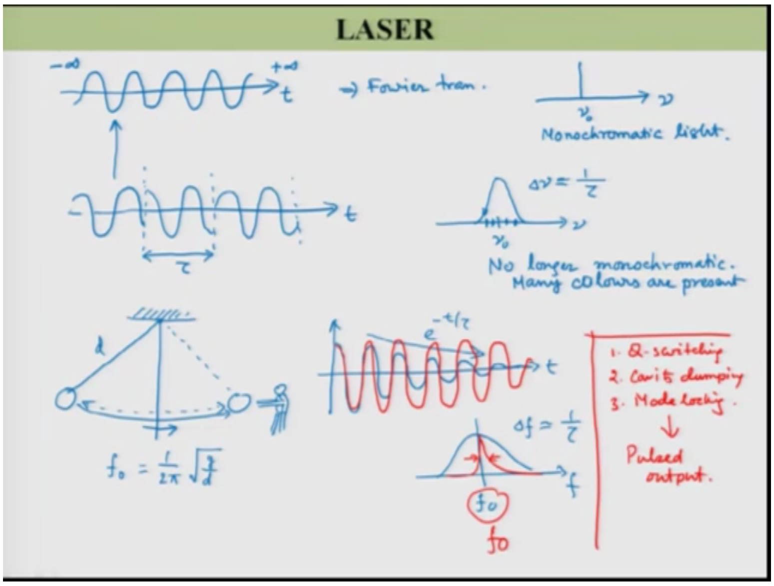

So, if I do the Fourier transformation this is in the time axis I will get in the frequency axis. So, whatever you will get? You will get single line of that; that means, if the laser if this electric field will oscillate forever, if this electric field will oscillate for ever then the color means frequency right then the color will be single color.

So, you got a monochromatic light right I got a monochromatic light. So, here monochromatic light you see the first property automatically comes in now the question is that why our normal light sources right like spectral lamp why they are not oscillating with constant amplitude and constant frequency forever right. The reason is that those in that case the emission are just nothing but the atomic emission for this spectral lamp. So, the emission is coming from the molecules or atoms present in this spectral lamp and there had is different life time and the emission is coming from the excited state species of those atoms. So, the emission is coming as a blast.

So, the emission is present for some time and then emission is not there, then for the next time the emission is coming from the other atoms right present in this spectral lamp. So, if I draw this kind of emission of spectral lamp what you will going to see is let us say amplitude is constant like this phase is constant time, but it finish over here that atom stopped emitting. The next a atom is start emitting, but that electric field is somewhere here and then is finished.

The next one probably will be like this and then finishes then you have many many such kind of atoms right. In this case if the duration of this one cycle for one atom the electric field is sustained for tau, this tau is not infinity in this case. So, if it is sustained by tau otherwise the amplitude is constant here right, then if you do this Fourier transformation what you will going to see in that frequency domain, it is not a straight line is not just a single vertical line, but it has some width and that with this proportion almost equal to 1 over tau right is almost equal to over tau; that means, here it is no longer monochromatic it is no longer monochromatic; that means, I have different colors over here many colors are present right here this means one color, this means another color, this means another color, this in another color.

On the other hand if I haves this system if I can make it like that way, it will keep on oscillate right then what you will going to get the transformation from this high bandwidth to very small bandwidth so monochromaticits. So, I have to do something so that this system will oscillate forever; and that is done by this light amplification method right. So, just give you a simple example for that which we have seen in our real life. So, if you look at the pendulum.

So, this pendulum will oscillate like this and its frequency is equal to 1 over 2 pi root over g by d. If this pendulum is present in a friction less environment there is no friction here and there is no air friction this then this pendulum will oscillate with this particular frequency forever right then I will get a perfect oscillator for this, but as you know that because of the friction the amplitude of this pendulum will going to decrease as a function of time.

So, let us consider the amplitude was like this and then this pendulum is decreasing decreasing and ultimately it will going to stop. So, damped oscillation right and if this decay I can write as e to the power minus t by tau. So, this is my time axis right then the Fourier transform of this will be something like this, the central frequency will be f 0, because I have written at the f 0 over here, and then delta f will be almost equal to 1 over tau.

Now, if you want to make a clock out of this pendulum the ancient clocks right earlier days right the clocks were made based on this frequency observed in this pendulum right. So, then this f 0 has to be unique only one value otherwise this clock will not run we clock will not give you the correct time. So, for this f 0 has a particular value what you have to do? Why have to maintain this oscillation right as a constant amplitude oscillation forever; because of this oscillation this pendulum there is a friction with the air. So, and for each oscillation for each time the this ball will come from here to here right the amplitude is decreasing little bit because of this friction with the air.

Now, if you stand here and push this pendulum little bit, why you are doing so? You want to overcome the loss right if you can do so, then this pendulum will keep on oscillate with this constant amplitude. So, you are giving some sort of energy which is used to overcome the loss, right then this oscillation will be something like, this because you have overcome the loss. So, if you take the Fourier transform of this oscillation this will be like this. So, here delta f is much much smaller, because in the decays much much smaller this almost constant amplitude. So, you will get unique value of f 0 in this case, here in the other case the f 0 value changes it consists of different values it has a delta f within the frequency.

So, in laser we do the very similar thing so that this loss of this optical electric field the loss of this electric field is not that much so it means that it is sustained for longer time right. Now in the lasers we have a different type of method to get a pulse laser right the first one is this Q switching. I am not going to describe all this method because this course is not for this if you take the course on lasers and its application, then probably you will see that how this keep switching or other process actually works to get the ultra short laser pulse. Second is cavity damping third is mode locking. So, these are the different methods by which one can get this from a constant amplitude oscillation to a pulse light output of the laser.

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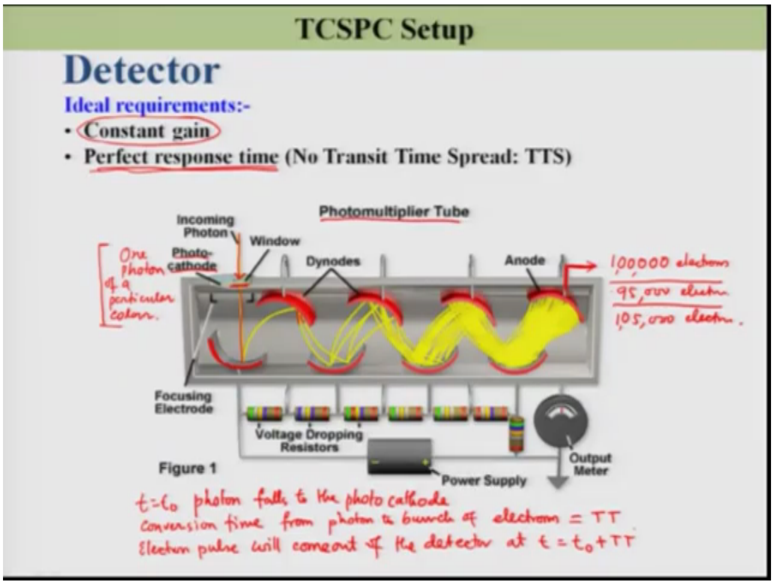

So with this let us move to our detector, because in this case just look at our this diagram. So, we discussed little bit about this laser and I said you our requirement and what is the reality, now I will going to discuss this part detector. So, what is our ideal requirement? The detector must have the constant gain here constant gain I will explain what it mean; and it should has a perfect response time. To explain these two let me show you the typical detector is a photomultiplier tube, as I said earlier that this is based on the photoelectric effect.

So, when incoming photon hits the photocathode over here, it rejects one electron and then this electron is somehow directed to this device which is called the dynodes and here this electrons is getting multiplied right, so from here 1 2, 2 to 4, 4 to 8 and so on and so forth right. So, ultimately in this anode you will get lots of electrons coming out of this anode which can be detected as a current or as a voltage whatever you want.

Now, the question is that if one photon falls over this photocathode, one photon of a particular color. So, here I am talking about one photon particle nature color wave nature right. So, one photon of a particular color is falling on this photo cathode, then let us consider that I will get 1 0 0 0 0 0 electrons coming out here. What is the guarantee that when the next photon with the same color right next photon of the same color will fall on this cathode exactly this number of electron will be ejected right there is no guarantee; if it is so, I call this as a constant again. Every time one photon falls of the same color every time 1 lakh electrons is ejected from this anode, but there is no guarantee like that; that means the detector will not going to operate as with a constant gain it has a variable gain. So, in this case I have some time 95,000 electrons coming out; sometime 1, 05,000. So, coming out and so on right that is the problem why I will tell you later. Another thing the detector must have a perfect response time what I wanted to say is that say at time; t equal to t 0 the photon falls on this photo gathered by two, here t equal to t 0 photon falls to the photo cathode; obviously, from here to here it will take time because none of the process is instantaneous it will take something. Let us say that time taken to from this photocathode to this anode rejection of this bunch of electrons it is let us say the t t that is the transit time transit time.

So, conversion time from photon to bunch of electrons let us say I call this as transit time TT. So, when this electron pulse will come out of these detectors. So, the electron pulse will come out of the detector at time t equal to t 0 plus TT.

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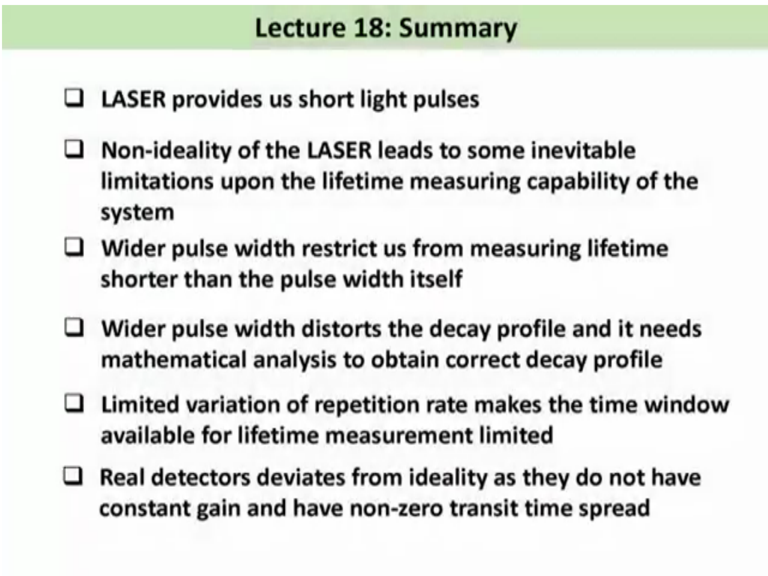

So, we will finish here and we will continue our discussion on the next lecture.

Thank you.