# Basics of Fluorescence Spectroscopy

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## Lecture – 19

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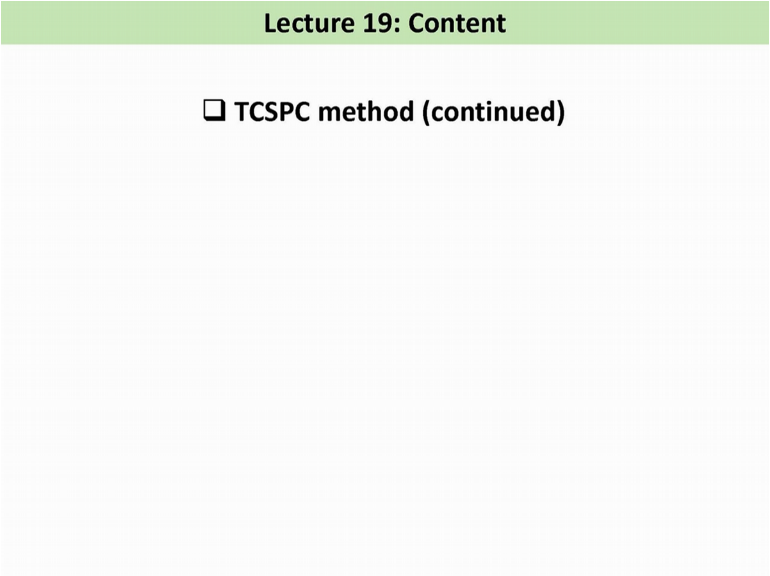

**Lecture 19: Content**

❑ **TCSPC method (continued)**

Welcome to the lecture number 19. So, in the last lecture we are discussing about the detector of this TCSPC system. So, as I said that the ideal requirement is that constant gain of this detector and the perfect response time. I have already discussed about the constant gain. Now I will discuss about this response time of the detector.

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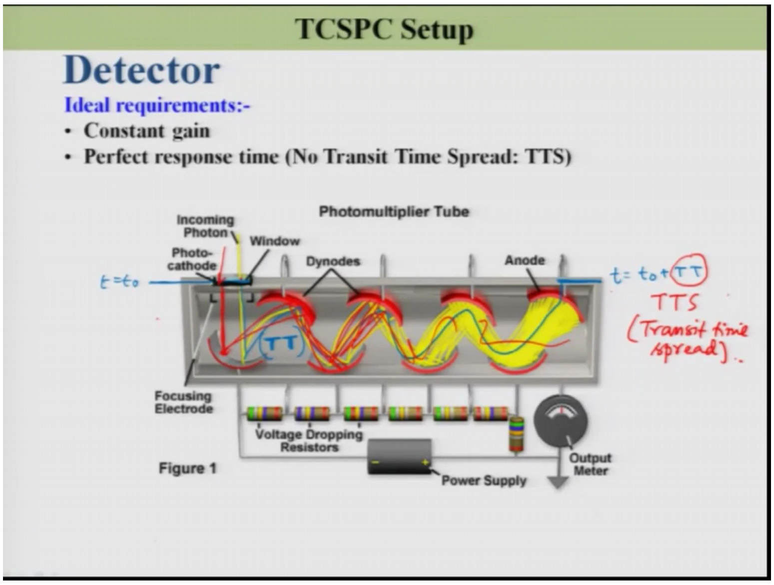

TCSPC Setup

**Detector**

**Ideal requirements:-**

- **Constant gain**
- **Perfect response time (No Transit Time Spread: TTS)**

So, to do that let me consider that the light the photon when interacted this photo cathode the time is time t equal to t 0. So, that this time is time t equal to t 0, and the whole process starting from here all these electron multiplication all these, these, these, these, all these complete process right obviously, this is not instantaneous I said. So, this process let us say is will take a time TT this is a total time taken for this process.

So, here right at what time this electron pulse will come out at time t equal to t 0 plus t t, but it is no problem for me because I will simply add this TT during my time measurement no problem at all, but now if this photon is falling because this photo cathodes having some dimension, this is the dimension of this photo cathode. See here this is the dimension of my photo cathode.

So, the photon may fall at this position as shown here, but it may also fall in this position because it has some dimension. If it falls on that particular position then this ejected electron will be directed around this position of this first diode, then this will be directed like this way which are will be directed like this and then all these, these, these, these, things, which you can immediately figure it out from this diagram that the path travel for this red one and the path travelled for this yellow one they maybe different maybe same is true, but maybe different in most of the cases it will be different. That means, whatever I said this TT is not a constant value.

Whatever I said this TT is not a constant value that means, I have is spread in transit time that is called the TTS I have the transit time spread, which I am trying to show you in this slide.

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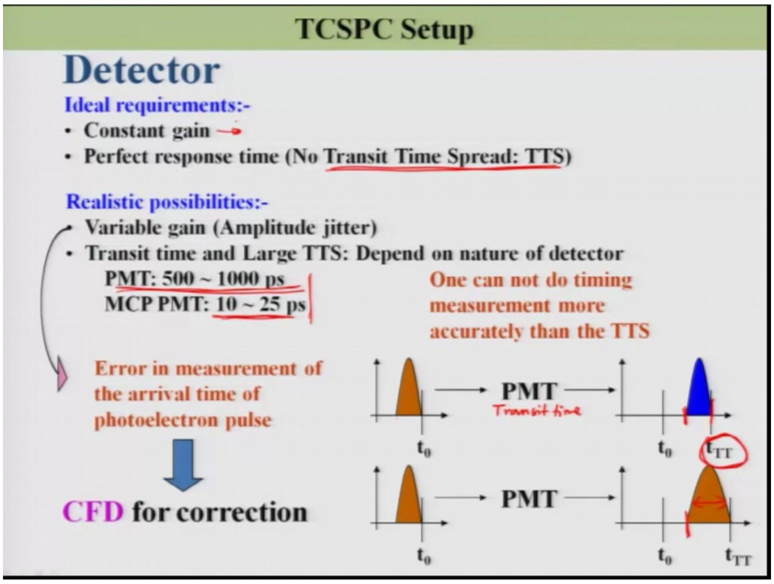

## TCSPC Setup

### Detector

**Ideal requirements:-**

- Constant gain
- Perfect response time (No Transit Time Spread: TTS)

**Realistic possibilities:-**

- Variable gain (Amplitude jitter)
- Transit time and Large TTS: Depend on nature of detector
  - PMT: 500 ~ 1000 ps
  - MCP PMT: 10 ~ 25 ps

One can not do timing measurement more accurately than the TTS

Error in measurement of the arrival time of photoelectron pulse

**CFD for correction**

Suppose this is my electron pulse which supposed to come at t 0, but because of this transit time of the PMT because of this transit time, I am going to get this at time TT T, but this is not a constant right this is not a constant because this width may vary. So, sometime I will get at this position, sometime I will get at this position and so on right. So, this is called the transit time spread and this transit time spread.

So, here I have written that and this transit time spread is the most difficult part of this kind of detectors and I want to measure the arrival time of the photon. If you remember our TCSPC system in the TCSPC system, I want to measure the arrival time of the photon the photon arrived here and it took some time for the conversion then the electron pulse will go and tell that stopwatch my TAC that now I got a photon. So, you stop counting.

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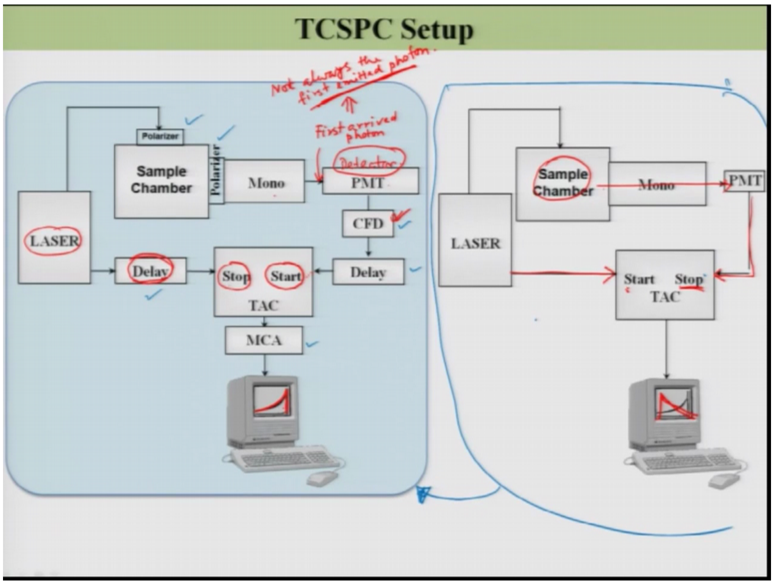

TCSPC Setup

So, when I have let us describe it once again over here. So, here when the laser has excited your sample in this case, it send one signal that you start the counting the time and then there is a emission of photon from the sample and then the first emission photon is being detected by this detector, these detector will send the signal, now I got a photon so you stop counting. And then that is the time between the excitation and the emission of a photon, and we plot such kind of event millions and millions times to get the histogram you remember.

Now, in this case what will happen that from the excitation and the detection of the photon there will be a time error and the time error is originating from this transit time spread as I explained in the last class, but these value of the transit time spread if it is small like 1 picosecond, 2 picosecond then I have no problem, because I am going to measure a (Refer Time: 06:16) of nanosecond if there is a error of one picosecond like a 1 by 1000, then who cares nobody will care. But unfortunately for normal detector the value of this transit time spread is really high is about 500 picosecond, 1 nanosecond if the detector quality is bad then it could be 1 nanosecond is a transit time spread.

That means, you are going to measure 1 nanosecond lifetime and your error in the measurement is 1nanosecond then you are no hair you cannot do the measurement properly. So, there are a lot of developments for this because this is the heart of this instrument right is a detector. So, detector is detecting the photon and it is converting to the electron pulse and that is used to stop the counter my TAC. So, there should be no error in this case otherwise whatever the error will be present that error will be reflected in the lifetime measurement. So, I want to minimize the time measurement error in this case.

So, for the normal PMT that error is about as I said 500 to 1000 picosecond and you cannot use such kind of detector to measure a lifetime of one nanosecond right. If the lifetime is 10 nanosecond, 20 nanosecond 30 nanosecond then is then no problem, but if it is less than 1 nanosecond 1 nanosecond then is impossible for you to measure and there is a lot of development how to reduce this transit time spread from this then and people have a ultimately come up with a better PMT, better detector where the transit time spread could be 10 to 25 picosecond, and nowadays people are using such kind of detector.

But what about if I want a detector whose transit time spread is less than that 10 or 25 picosecond there is no such kind of detector available in this world. The minimum time the transit time spread could be achieved like a about 10 picosecond, you cannot go beyond the 10 picosecond; that means, if you want a measurement of a fluorescence lifetime that lifetime is 1 picosecond if just consider that a lifetime is 1 picosecond with this kind of setup you will not be able to measure it, you have to go for another setup and that is why I told you earlier that I will going to discuss two setup one is the TCSPC which is widely used, and the another is the fluorescence of conversion.

The fluorescence of the principle of fluorescence of conversion is a completely different than this TCSPC, and in that case you will be able to although the detector will be same, but still you will be able to measure the fluorescence lifetime of one picosecond or something like that with this same detector, because the principle is completely different we will going to discuss that later.

Coming back to this a detector of this TCSPC so as you have understood that this a transit time spread is one of the important issues. And if you want to measure this lifetime with a better resolution, then you have to go for a better detector where the transit time spread is small. Another thing which I already said in the last day is that constant gain.

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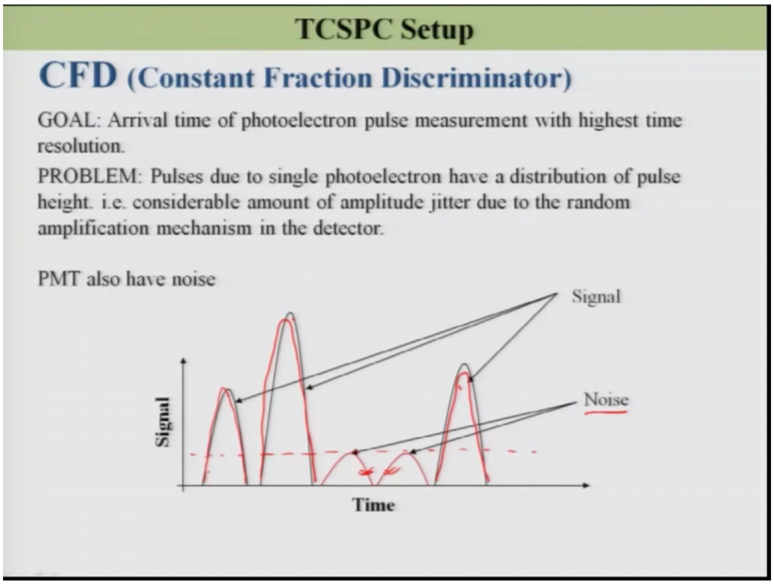

TCSPC Setup

**CFD** (Constant Fraction Discriminator)

GOAL: Arrival time of photoelectron pulse measurement with highest time resolution.

PROBLEM: Pulses due to single photoelectron have a distribution of pulse height. i.e. considerable amount of amplitude jitter due to the random amplification mechanism in the detector.

PMT also have noise

Let me show you this with this diagram. So, I said that the detector they do not have this constant gain; that means, if one photon falls sometime 1 lakh electrons will ejected sometime 95,000, sometime 110 1000 electron will be ejected and so on. So, there is a amplitude jitter; the signal will sometimes small sometime large sometimes small and so on and so forth.

Now, you also have this noise that I already talked about during the absorption spectrophotometer, that all the detectors as a thermal noise. Even the photon will not form the detector will give you some signal, those are simply the thermal noise. So, these are the let us say the thermal noise. So, if I want to get that timing of the arrival of the photon then I should not detect these signals because these are the noise. So, what I should do? I should put a threshold electronically over here, and say that if this signal is more than this noise then those are my signal otherwise they are not signal those are noise I will not going to consider those as a signal.

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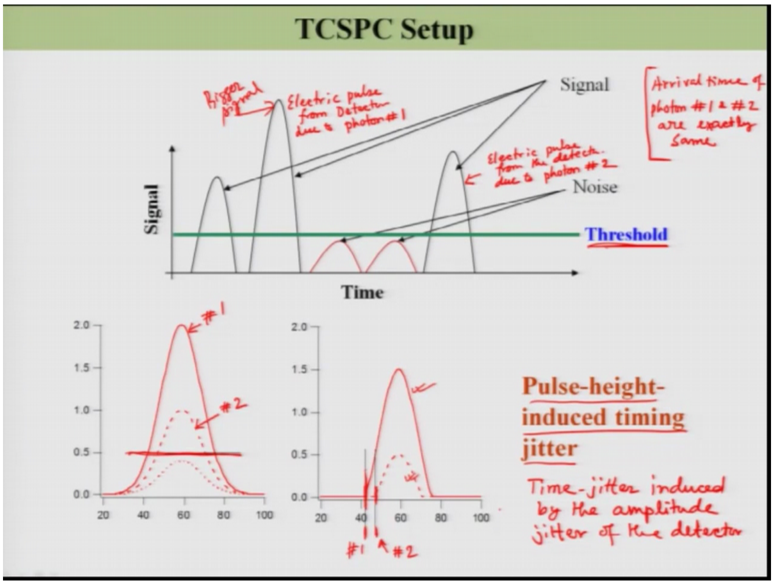

**TCSPC Setup**

Arrival time of photon #1 & #2 are exactly same

**Pulse-height-induced timing jitter**

Time-jitter induced by the amplitude jitter of the detector

Now, you see that when I put this kind of threshold over here and I am just showing it over here by a Gaussian type of a pulse. So, I am now putting this threshold over here. So, here in this a axis this is my start time of this signal the big signal, and this is the start time of this small signal. You remember here that both of these pulse this is let us say this is I am writing it like that way, this is the electric pulse from detector due to photon number 1, this is the electric pulse from the detector due to photon number 2, and I consider here that photon number 1 and number 2 the arrival time arrival time of photon number 1 and number 2 are exactly same I consider this.

So, when they arrived exactly at the same time, now I am not considering this transit time spread I am considering that amplitude of this signal right because they have a variable gain. So, in this case for the photon number 1 the gain was more than the photon number 2. So, I got a bigger signal for photon number 1 here bigger signals, and I got a small signal in the case of photon number 2.

So, this is number 1 and this is for number 2. So, if they arrive at the same time then these two signal will should reach the detector exactly at the same time, but because of this threshold and because of this cutting, you see the start time of this signal is here and the start time of this signal is here. So, this is for the number 1 and this one is for number 2. So, their arrival time to the detector the arrival time of the signal due to photon 1 and photon 2 they are different arrival time are different and this is a big problem.

Although these two photons arrived at the same time, but their they arrived at the same time to the photocathode, but their arrival time to the stopwatch the signal arrival time to the stopwatch are different. This is known as no I have written here pulse height induced timing jitter there is a timing jitter right what is also known as time jitter induced by the amplitude jitter of the detector.

So, we have a problem over here how to solve this problem? And this time is a big time big time gap it is not small is about 200 300 picoseconds sometime 500 picosecond that is compatible to your lifetime measurements. So, if you do not take this problem seriously then your lifetime measurement will be too much erroneous right. So, I have to somehow solve this problem, and the way to solve this problem is a introduction of the CFD just after the PMT let me show you over here.

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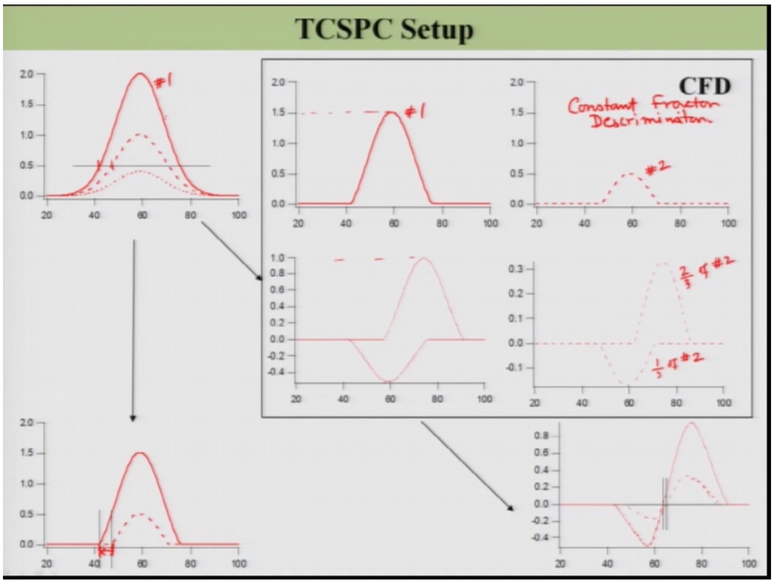

TCSPC Setup

CFD

The CFD is a device is a electronic device right is a device by which I can reduce this difference in time to a smaller value it cannot be zero, but it can be reduced significantly. So, just I have done a simple a thing for to show you the function of the CFD, in the CFD like after this threshold I got the two pulse one arrival time is here the second arrival time is over here.

So, let us take this first one the bigger pulse the number 1I took this number 1 over here and I have divided this number 1 in two different pulse one is 66 percent, here they see the amplitude is 1.5 now I have made it 1 and another is 0.5, the smaller one is inverted and shifted in time at your desire. And then these two you sum up; if you sum up these two what you will going to see you will see such kind of profile right as shown here hope we will be able to see. So, this kind of profile like this kind of profile, for the smaller one you are you do the same thing you divide this as a one third, two third, the two third is over here, this is for number 2.

So, this is two third of number 2, this is one third of number 2 inverted shift at the same time and then you sum it up and you will see this curve right as I showed here this curve. And now you see when these two pulse will get a positive value the time difference is less than the time difference which was present or a originally. So, in this case a by using this kind of CFD, I can reduce that time jitter induced by the amplitude jitter of the detector, and the full form of CFD is constant fraction discriminator.

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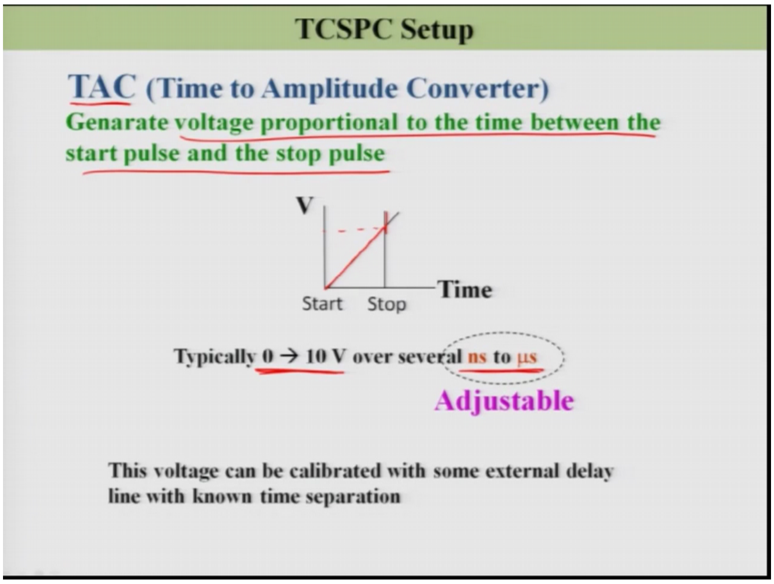

**TCSPC Setup**

**TAC (Time to Amplitude Converter)**

**Genarate voltage proportional to the time between the start pulse and the stop pulse**

**Typically 0 → 10 V over several ns to µs**

**Adjustable**

**This voltage can be calibrated with some external delay line with known time separation**

So, now let us discuss about this time to amplitude converter TAC. So, as I said earlier that this is a kind of stopwatch, but is actually is based on the capacitor right. So, it generate a voltage proportional to the time between the start pulse and the stop pulse right. So, when it comes as a start pulse. So, the voltage will keep on generate it will be charging and once there is a stop it measured this voltage and there is a calibration that how much voltage can be generated with how much of time. So, with this calibration like typically 0 to 10 volt can be generated with several nanosecond to microsecond time region. So, I can divide that per a millivolt is how much time, and that will be used for the measurement of the time like it will behave like a stopwatch.

With this what we understood till now is this set up is this set up. So, I have a laser it excite the sample the desired emission wavelength is selected by this monochromator, and then this photon the first detected photon is detected by this PMT. So, here note that here the first arrived photon which not necessarily the first emitted photon that is the heart of this TCSPC. If it is like that then there is a no decay always I will get a one count or several counts on the very first few time regions.

So, we will I will never get the count in the later time. So, I will never be able to reproduce the decay profile in the histogram. So, note that. So, not always the first emitted photon is the first arrived photon; that means the first detected photon. So, it is detected and now detector has amplitude jitter which will going to give you the time jitter and that is rectified by this CFD over here constant fraction discriminator and detector is also having the transit time spread which obviously, cannot do anything you cannot only have to buy a better detector where the transit time is very small and then it will use as a stop here. But here you notice here that I have just changed that start and stop like this way I have written here is top and here is start. So, it is the correct one right this is the wrong one let me explain it.

So, the reset time of this TAC is about millisecond and I said that the detection is very very small rate because I have to ensure the first emitted or first arrived photon should not be always the first emitted photon from the sample this is important. To ensure that what I said that I will reduce the detection rate to 2 percent or less than 2 percent so that the photon the first photon which is emitted like here it will go to some other direction it will not go into the detector if my detector is present here. So, the first emitted photon may go this way the tenth emitted photon may come like this way.

So, the photon which is emitted at much later time if you find a possibility that this will come towards the detector, then the detection rate should be very very low that is the important part in the TCSPC system. So, in that case as I said that the detection efficiency is generally reduced to 1 percent 2 percent. That means, for every 100 excitation I will going to detect only one photon; that means, you are going to excite, you are going to start the stopwatch the TAC 100 times, but you are going to stop it only once that will going to give me the overloading of the TAC, and the TAC reset time is also very slow.

So, better if I can use the emitted photon as the start and the excite and the excitation as the stop then only when there is a emitted photon then the stopwatch will start and then eventually it will be kind of right. So, in that case I have it like this way, this one is actually starting that TAC, but there could be some problem related to that these signal will come early than this signal that is why I have a delay here electronic delay. That somehow it delayed so that this signal is coming first and this signal will coming later. So, then the histogram will looks just a mirror image of what I showed in this case the histogram is like this in this case the histogram will looks like this.

Let me finish here and will see that if some other topic should be discussed on this TCSPC setup otherwise we will start the fluorescence of conversion on the next class.

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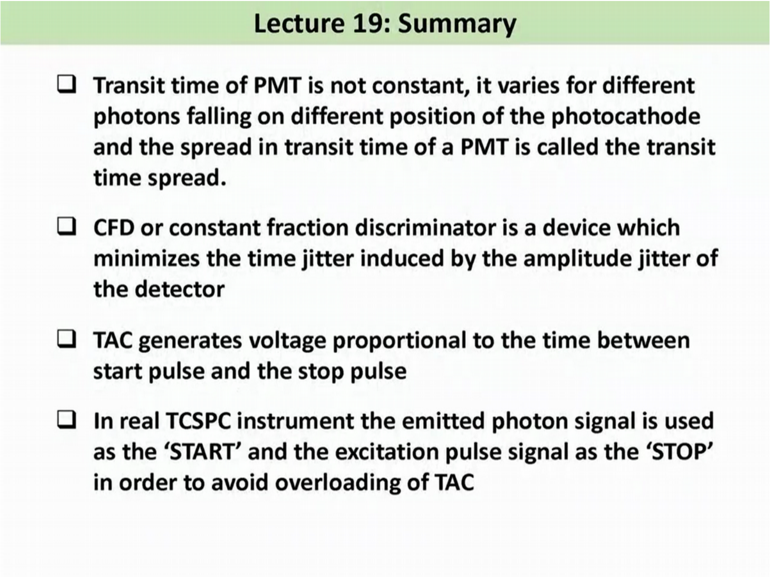

**Lecture 19: Summary**

- ☐ **Transit time of PMT is not constant, it varies for different photons falling on different position of the photocathode and the spread in transit time of a PMT is called the transit time spread.**
- ☐ **CFD or constant fraction discriminator is a device which minimizes the time jitter induced by the amplitude jitter of the detector**
- ☐ **TAC generates voltage proportional to the time between start pulse and the stop pulse**
- ☐ **In real TCSPC instrument the emitted photon signal is used as the 'START' and the excitation pulse signal as the 'STOP' in order to avoid overloading of TAC**

Thank you very much.