Ultrafast Processes in Chemistry
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Lecture # 07
TCSPC for Picosecond-Nanosecond Time Domain (Contd.)

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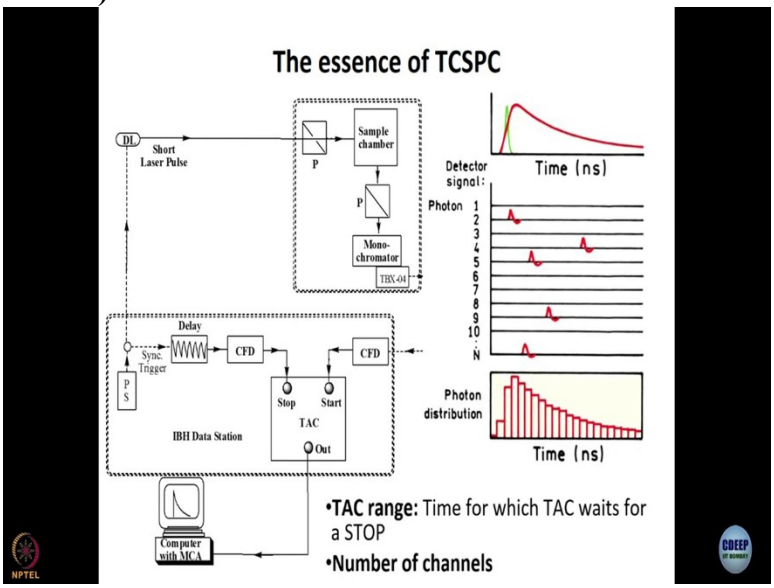

The previous module were studying the schematics of this is TCSPC or time correlated single photon counting experiment, this is where we were. Let us go a little further ahead today. And before we do that, let us revise what we have learned this here is the essence of TCSPC. Essentially, you are trying to record the difference in arrival times of a start and a stop signal. And you are trying to plot a histogram. The histogram that we are talking about in the previous module.

And what lies at the heart of the TCSPC is that this histogram that you construct is actually of the same shape as this fluorescence decay that you are trying to construct. Now, let us get into a little bit of nitty gritty of the instrument because the entire purpose of this is that we should be able to use the instrument when we do it. Of course, you might understand a little better when we see the instrument for ourselves when we go to the lab, but we need some preparation for that as well.

Now, there are certain terms that will need to know first one is TAC range is essentially the time for which a TAC waits to get a stop signal before resetting and starting all over again. So, you can think that the TAC range is the maximum time measured in your experiment. So in this decay that you have, you can think TAC range is the full scale on x axis on time access, typical smallest value of TAC range is that I know of is 26 nanosecond 50 nanosecond is more common.

What kind of TAC you will use depends on what kind of decay you are looking at, because for a good analysis of the decay it must be complete. So, if your lifetime is, say 5 nanoseconds, then well you remember the decay law right, I t = I 0 e to the power – t by tau or I t = I 0 divided by e to the power – t by tau . So what happens when t = tau it becomes one. So, I at time t = tau,is equal to I time 0 divided by E.

What is the value of e approximately 3 we can say 2.7 approximately 3. So very roughly, we can say that decay is in 1 lifetime, the signal or population, whatever you want think decays to 1 third of its value at time zero . Right I as time t = I as times 0 divided by approximately 3 which means fluorescence intensity will decrease to about 1 third of its value in 1 lifetime. So which value will decrease in three lifetimes not 0 is the correct but useless answer. Yes, 1 lifetime 1 third.

Let us go step wise two lifetimes yes 1 by 9, 1 third or 1 third 3 lifetimes 1 by 27, 4 lifetimes 1 by 81, 5 lifetimes so it belongs very small. So typically you want to keep your TAC range to about five times of the lifetime. So, 50 nanosecond TAC range is fine, provided your lifetime is no longer than 10 nanosecond. At the same time, you do not want to keep the TAC range very large. The lifetime is one nanosecond keep TAC range, range of 500 nanosecond. Then what will happen? One nanosecond. So becomes practically 0.

So, for 200 - 5 195 nanosecond, out of that 200 nanosecond, you are going to be recording 0 it will waste time so is important to choose the correct TAC range when we say we are choosing correct TAC range what are we actually doing? Setting the TAC window, letting the TAC wait for a bigger signal right or we can say that see the maximum signal that we can usually get is 10 volt. We are saying that when TAC range is 50 nanosecond.

Then 10 volt signal is equivalent to 50 nanosecond when that range is 100 nanosecond that the same 10 volt signal is equivalent to 100 nanoseconds. What does that mean? What is this 100 nanosecond? Delta t remember, time of charging? So what we are saying is for different times of charging, we are keeping delta V to be same. How do we do that? The voltage remains same of the capacitor that the capacitance is not the same. You have somehow changed the capacitance.

How do you change the capacitance? Of course, nowadays, everything is inside a chip. So god knows how they do it. But if I go back to the good old days of components, electronic components, then what you actually have to use is a variable capacitor. Has anybody seen an old fashioned radio set where you actually turn a knob and go from one station to the other? What are you doing there? You are changing the capacitors in an LCR circuit.

That is why their characteristic frequency changes. And in conventional capacitors, what happens is you have this plates that go into each other like this. If you bring them out, of course the plates are not like my fingers, you can think that my fingers define the outer edge of some circle. And then like this, maximum overlap of areas, if you bring them out that overlap decreases and capacitance decrease.

So you can use a device like that to change the capacitance and therefore TAC range the point I am trying to drive home that this is not magic, this is not for good. You can understand the principles by a high school level modern physics that everybody has studied. Second thing that is important is number of channels. Remember, TAC range it sounds foolish if I say it like that, but at TAC range is something that is associated with TAC. Sounds like stating the obvious.

But the reason why I am saying it is that it is important to remember the number of channels is not associated with TAC it is associated with multi-channel analyzer it is very important to use the correct number of channels also because suppose I have 50 nanosecond TAC range and our 1000 channels you never have 1000 channels, it is always in some binary number but let us for convenience that let us say 1000 channels then what is the time resolution what is the time per channel.

One channel is each channel is a point remember. So, what is the resolution I have 50 nanosecond full scale thousand points quickly 50 picosecond 1 nanosecond is 1000 picosecond so, it is actually easy, now I am saying instead of 1000 channels, I have say 5000 channels then what is the resolution, earlier it was 50 picosecond per channel when number of channels was 1000. Now, let us say I increase the number of channels to 10,000 then what is the resolution.

5 picosecond per Channel , now, the question is how many points will I use? Should I use 1000 points, should I use 10,000 points again the answer to that depends on what kind of decay you are looking at suppose, a typically you should have 100 points per lifetime. So, if you are estimated lifetime is 1 nanosecond, then 1 nanosecond divided by 100 how much is that 10 picosecond, 10 picosecond per channel is a good resolution.

But, if you are lifetime expected is 5 nanosecond, there is no need to use 10 picosecond per channel. You can use 50 picosecond per channel right. Why is this important because if you use a resolution that is not good enough, then you do not get a good decay. If you use a resolution that is unnecessarily good, then you waste time. Remember how the experiment is happening in every point in every channel counts are being increased.

So, a poorer resolution essentially means 2 or 3 or 4 or 10 or 100 channels have been merged, so it goes up faster. So, it is important that we use, not maximum not minimum, but optimal number of channels for your experiments. Why because you do not want to spend an entire lifetime doing an experiment. But you do not want to do an experiment whose data is not reliable. How will you know exactly what number of channels to use we discussed an example a little while ago?

This comes for example, from experience. And experience comes only when we do experiments with our brains switched on. If you do experiments with brain switched off, there is no experience that is gathered, even if you do the same thing over an entire lifetime. So it is like driving a car, like swimming. Once you learn if you do not think you just do it right so, that experience has to come.

So, initially when you do the experiment, you have to do it consciously. And you have to do rough experiments in the beginning to understand what kind of time constants you expect. Because you are not going to know right looking at the sample you cannot tell whether the lifetime is 100 picosecond or 5 nanosecond. So limited experiments are important. Thinking is important experiences is important. Now we move on to.

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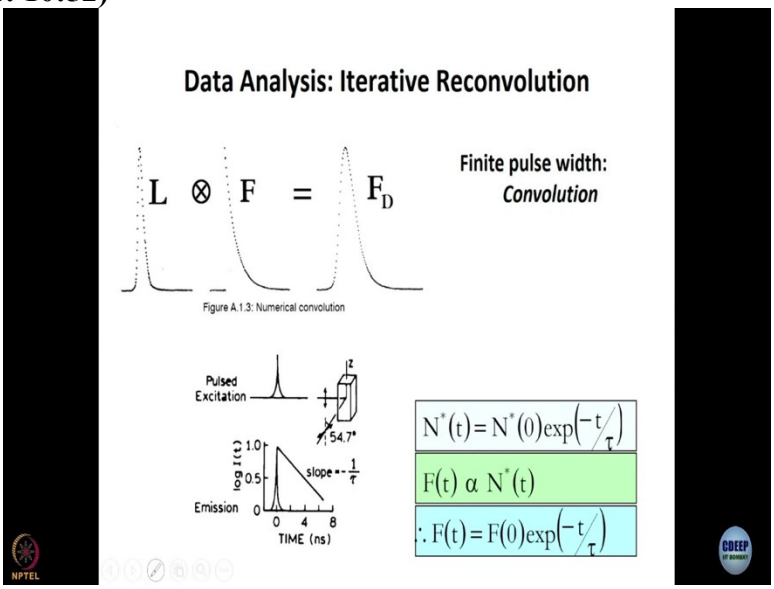

rather important question of data analysis. You got the data fine. How do you fit it? How do you get the data? We have already discussed the fitting model, most commonly use fitting model is single exponential multi exponential. Later on, we'll talk about some more fitting models also. But now, our problem is that we do not have the ideal situation where we are exciting with a Delta pulse, we have a pulse of a finite width.

So, what we get F_D is really a convolution of the instrument function, laser pulse as instruments sees it you can think alpha laser and the actual fluorescence decay F. Convolution means a mixture, a hopeless mixture that cannot be separated easily. We will see what it actually means graphically. It is important to understand that the graph you see is not what the one we want it is the one we want mixed with the laser pulse as the instrument sees it.

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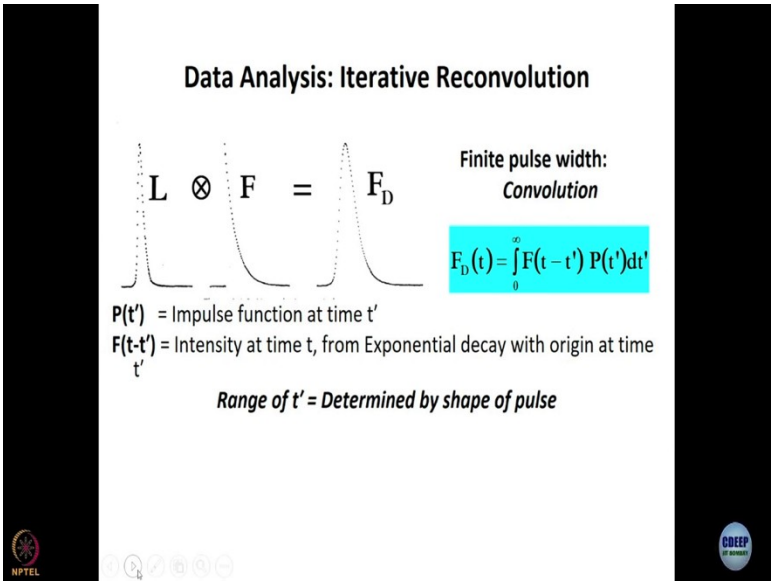

So, this is what is called the convolution integral. This is what we actually get

$$F_D(t) = \int_0^\infty F(t - t') P(t') dt'$$

This is convolution integral. And for the uninitiated, this I am sure means nothing. If you are very good in mathematics, well as you can see this and make sense of it, but let us not take a chance. Let us try to see what it actually means.

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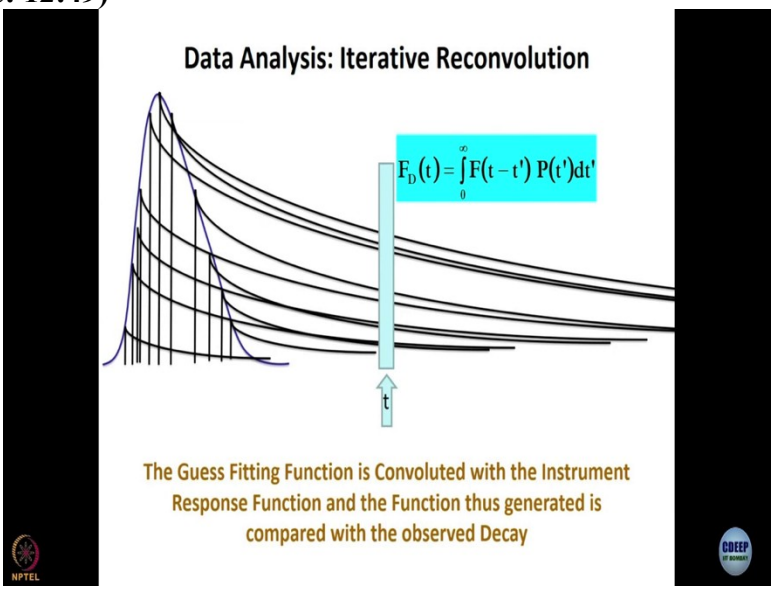

Let us think of a laser pulse of a finite width. So we can think that every point on this envelope is the tip of a delta pulse right and how many delta pulse would be there under this umbrella in principle in finite in theory and finite in practice, the number will actually be finite because as we

discussed already we work with some picosecond per channel resolution. So, depending on how many picoseconds are covered under this curve.

Suppose, we have hundred points here, then we are going to get hundred Delta pulses experimentally, the number is not infinite, it is determined by what kind of time resolution we use. Let us see the effect of 1 such Delta pulse. Let us take a small 1 at initial time. This delta pulse. Well, what I have done is not a delta pulse. This is a Delta pulse. What I am saying is that is going to you rise to decay like this.

What about the next delta pulse that will also give rise to another decay like this whatever the next delta pulse same and this goes on right this is what the meaning of the deconvolution is ok . all these Delta pulses under the instrument function gives rise to a decay if you look at any time t the fluorescence intensity is given by a sum of all these fluorescence intensities from all these decays at that particular time that is the meaning of the convolution integral. Let us if you understand it a little better now.

If you see this arrow this is the time t we are talking about and we are saying that the Delta pulse occurs at time t dash. So, what is the time for which the decay has actually taken place we are measuring at some time absolute time t and the decay is the Delta pulse is at some time earlier time that t dash. So, for what is the time for which the decay is actually taking place as a result of this delta pulse t - t dash that is very easy.

So, that is the time for this particular Delta pulse. So, if I took any other value of this t dash this t – t dash would have changed. Now, see, this amplitude here or intensity at time t 0 here will let us call it P t dash. So, what will be the intensity at time t. F is a fitting function remember , e to the power - t instead of e to the power - t now, we are today it is about - t - t dash because the delta pulse is at t dash. So, t - t dash is effective time.

So, if is just single exponential decay t e to the power - t - t dash and P t dash is the intensity at time well t = t dash here go it. So, now, we come back to this integral what did you say the intensity is going to be given by the sum of the intensities that occur as a result of all this delta

pulses. So, if you write the most general expression, what is the range of t dash from 0 to infinity right so, that is the integral.

See F t - t dash multiplied by P t dash. Summation is replaced by integration integral 0 to infinity but 0 to infinity for not t, but t dash. So that gives us the intensity that we actually see at 1 particular point of time important to remember that at one particular point of time, right, I have not written this for the entire decade, only at the value of P were the arrow presently is to get the intensity at this value. You have to work out this interactive right.

This is the meaning of convolution. Do not get scared by the integral sign and integral is just a summation. How do you actually do it? You do it by a method that is called Iterative reconvolution. So anyway, this is what it means to see we are adding all the intensities due to all the decay is coming come out the all Delta pulses and the sum is given by this integral how do i do it by a method call it Iterative reconvolution.

See, we are saying deconvolution we are talking about we have to do deconvolution because here we have a mixture of the decay as well as the pulse as instruments is it how do I deconvolute the easier way of doing so called deconvolution is to take a guest function. To assume that the lifetime is 5 nanoseconds the moment you assume that you know what F t - t dash is going to be. So, what you do is with the guess value as it is called the assume value, You can easily construct well not easily if you have to do it manually it will take time of a computer It is very fast.

You can construct the fluorescence intensities at every point and then you compare the graph you have constructed and the graph you have experimented got it. Of course you will not get it right the first time. So to do it again, that is very difficult Iterative reconvolution, because you are knowingly convoluting the instrument response function with the decay law that you have you think is correct. And Iterative because you will never get it right the first time.

You have to do it in several rounds or several Iterations. So I think we will stop here today. And next day we are going to talk about goodness of fit. Because it is very easy for me to say that we have compared the graphs, how will the computer compare computer does not have eyes. So,

next day we will learn first of all, what are the eyes of computer how does it know whether the fit is good or, fit is bad and then we will talk about some more decay models.