Ultrafast Processes in Chemistry
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Lecture No. 08
TCSPC Lab

Today we come to the lab and we have a look at our time correlated is single photon counting setup. In the setup that we are going to show you today, the light source is a femtosecond pulse titanium sapphire laser, whose output varies from 690 nanometer to 1040 nanometer. And then we have something called a pulse picker, which cuts down the repetition rate to the desired value. And after that we have second and third harmonic generation arrangements by which we can generate blue and ultraviolet light.

This light is used to excite the samples and then the fluorescence of the sample is goes through a time correlated single photon counting detection, detector and electronics and then regenerate the decay. So, today the purpose is to show you how actually decay is recorded and wherever possible the components of the instrument itself. First let us have a look at the laser.

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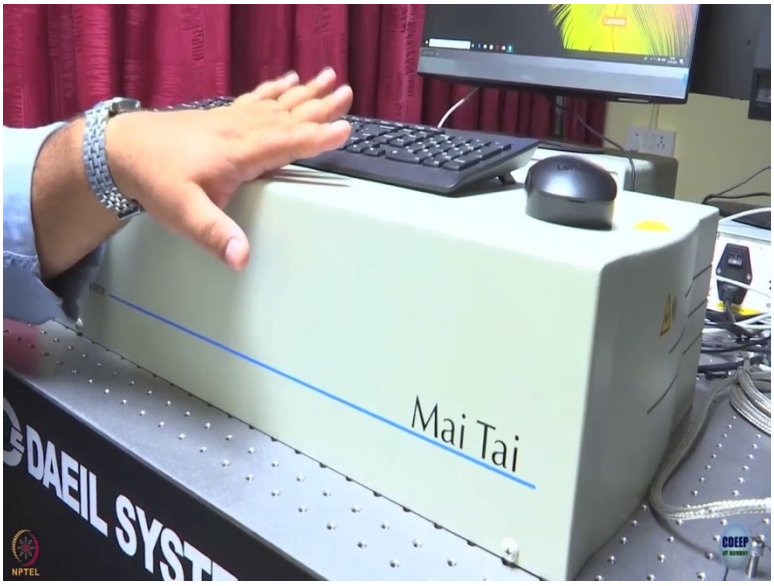

This is our femtosecond titanium sapphire laser. Now, unfortunately, this laser is sort of a black box below you cannot open it and show you what is there inside. We take a raincheck for that. And later on in an older model will show you actually what is there inside a laser.

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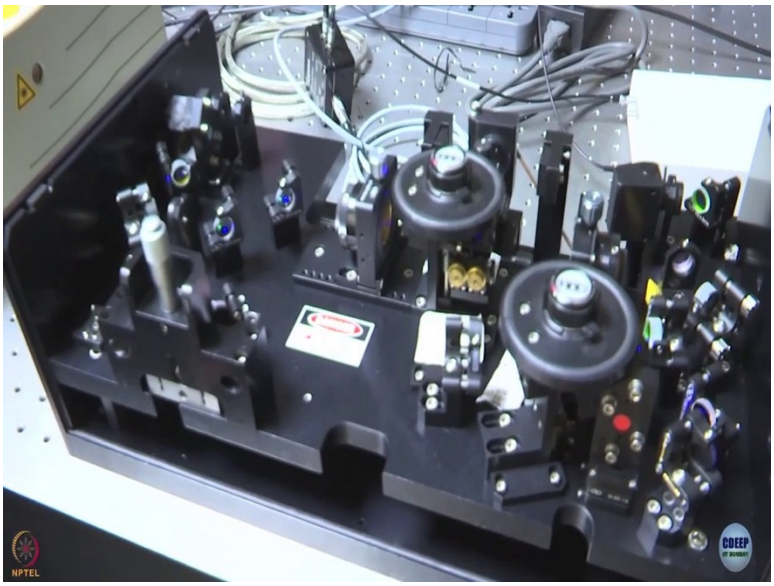

Now, the titanium sapphire laser gives us output as I said in the range of 690 nanometer to 1040 nanometer, which is basically red almost near IR kind of range. This wavelength is not really useful for excitation, if you are going to talk about fluorescence experiment. Secondly, the repetition rate at which the pulses come out of the laser is about 80 megahertz or 100 megahertz, you can think, which means the separation in time between two pulses is about 12.5 nanosecond.

And that is a problem because if we have a long decay, which does not get over in 12.5 nanosecond, then we cannot really record it. So, first of all, we have to cut down the repetition rate. And then we have to generate what are called higher harmonics, about which we will study in detail a little later on. First, let me show you what is there inside this box. So, what you see here is a lot of optics, right. And when you see for the first time, everything looks alike.

So, just to give you an idea of how things work, the light comes in from here, red light and this slab that you see, that is a pulsw picker. It is a quartz block on which we apply some radio frequency, which gives it a variable refractive index. And as a result of that, we can chop down the number of pulses. So, that depends on what kind of radio frequency we give that and then the red light comes and is incident on a crystal that is plased there you might be able to see a little bit of blue light there, that is where second harmonic generation takes place.

Second harmonic generation means you can think, 2 photons of smaller energy join up to produce a photon of exactly double the energy. Now, this conversion is never complete, maximum efficiency that you can hope to get is about 20%. So, out of this crystal, we get say 20% of light is converted to blue and remaining 80% remains red that comes here and gets split this is a dichroic beam splitter so now blue and red light take a different path and one of these is given variable time delay using this micrometer screw gauge. Then both of them travel by the same path on to a third crystal, which is kept here, that is a third harmonic generation crystal. In third harmonic generation, what we do is, we mix 1 photon of fundamental frequency and another of second harmonic and as a result, we generate third harmonic which is basically lambda by 3.

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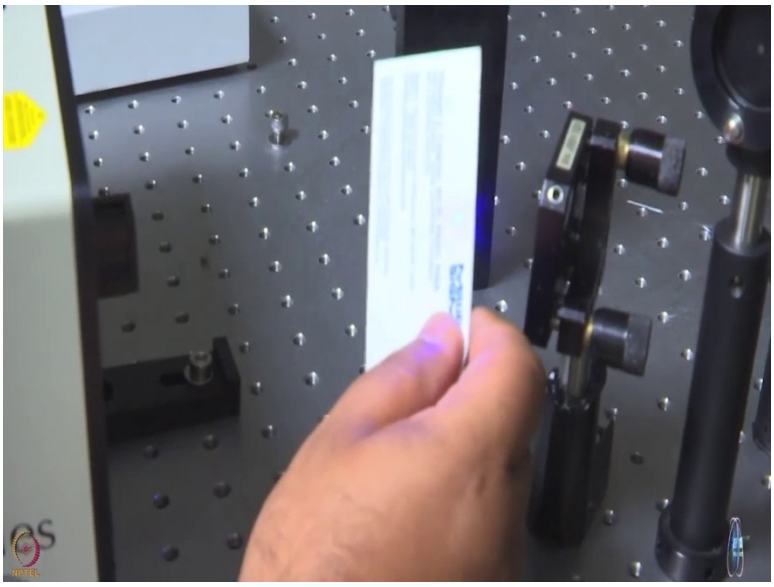

And now, it is time to show you the output. You might be able to see 2 blue spots here, the lower 1 the brighter 1 is actually blue. Now, we are working with fundamental frequency of 885 nanometer. So, the blue spot this one is half of that 442 nanometer approximately and the one the upper one which looks new to our eye is actually the UV light third harmonic 85 divided by 3 nanometer which is about 290 nanometer.

So it looks blue because the UV light is incident on the card which has a lot of proteins and stuff and they emit in the blue that is the fluorescence we see otherwise it is not visible to our eyes.

Now, we have to choose which one we want the blue one or the red one, right now the red one is being used.

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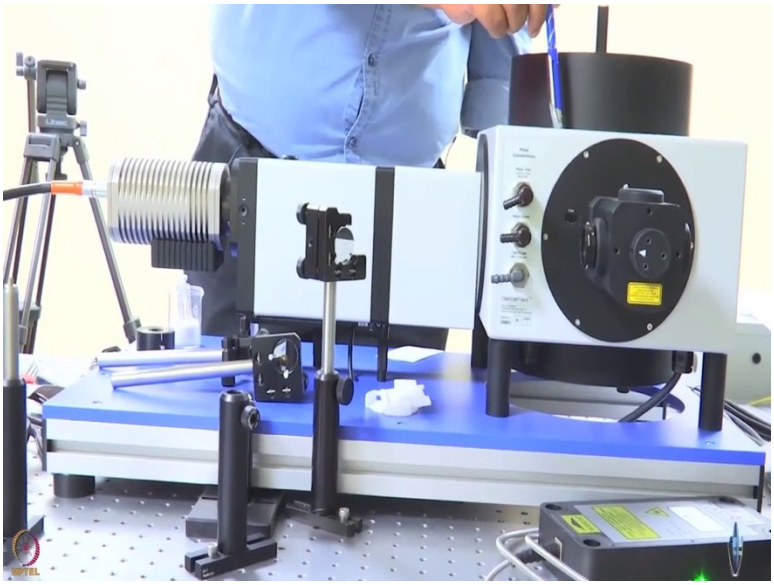

So the red light goes hits this mirror then comes to this mirror and gets deflected into the sample chamber where we keep the sample.

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So, we have kept our sample in this cuvvette and that goes in here. So, excitation light comes this way, hits the sample emits in all directions, but we record in a perpendicular direction.

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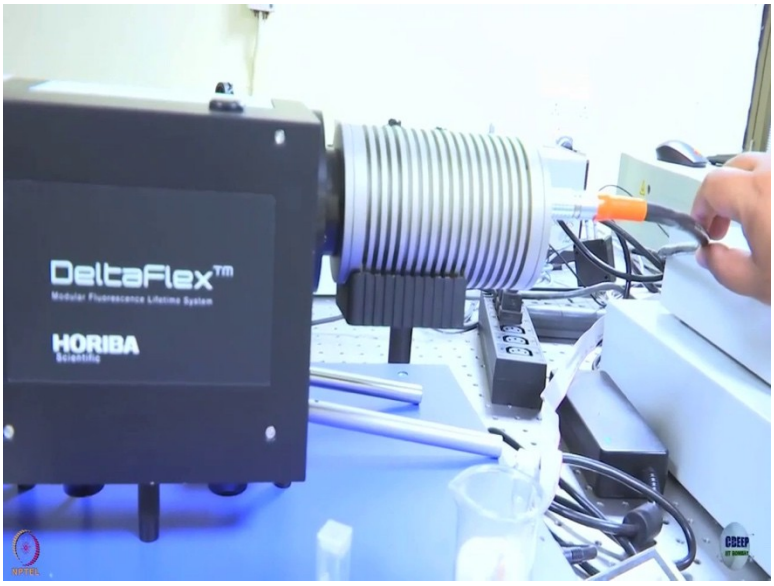

Let me show you the detection channels. The first piece of optics through which the emitted light goes through goes is a polarizer that is kept here. This is a polarizer put it in here we have a monochromator which we have discussed in the theory class and this is a fast detector that we have it is a new kind of hybrid detector and you can see the output of the detector goes through this thick cable.

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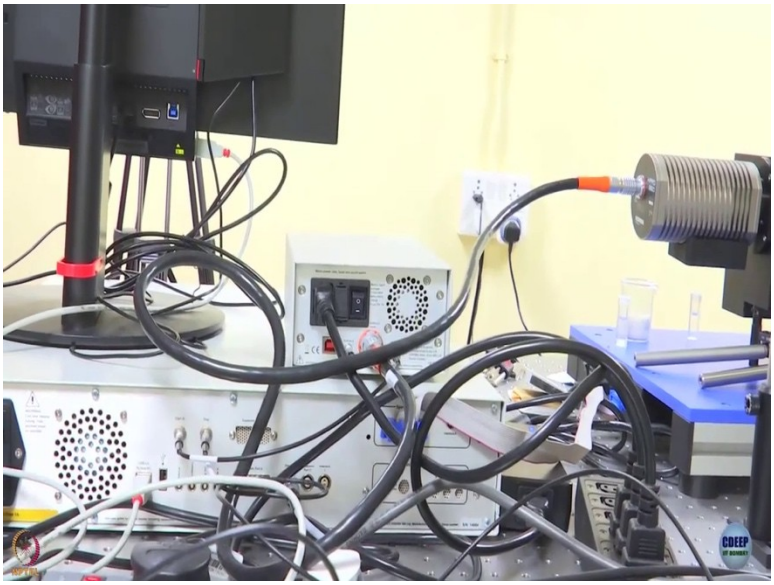

So, you see that thick cable it has come out and it has gone from a small box there. From there you might be able to see a small cable that comes out and goes into a terminal called start A that is the start of the time to amplitude converter that we have discussed in the previous class. And before going there inside that small box itself, we have the constant friction discriminator So,

that is start, what about stop you can see that there is another terminal there which is stop input from stop comes as a synchronous pulse from the laser power supply itself right now we are not trying to show it to you because it is a little too circuitous, but we do have a stop which comes from the source. So, here you see we are starting charging of TAC by the fluorescent signal and we are stopping by the synchronous signal. This is called reverse mode. And reverse mode is useful because, especially when you do a high frequency kind of measurement. Reverse mode helps decrease the dead time of the instrument and recording is fast.

Now, we will go over to the other side once again. And we will actually record a decay.

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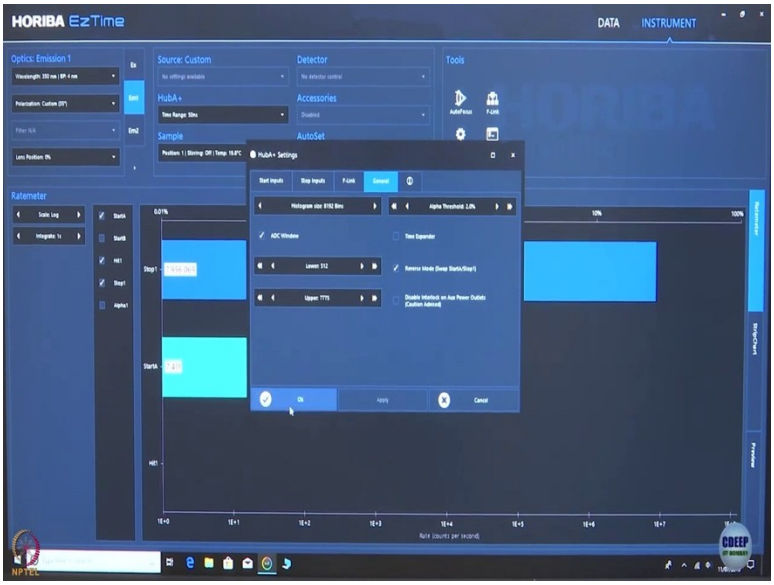

So here we are this is where we record the decay after the signal comes from TAC to the multi-channel analyzer and multi-channel analyzer is actually a card nowadays it goes inside the PC which acquires it for us in our case, all the electronics goes into the box that you see right here. So, on the screen, you can see 2 counts 1 is stopped and 1 is start, stop is something like 7956074 and stop is started about 7000 or 8000 or so, this gives you the repetition rate of the laser.

We are working at a repetition rate of about 8 megahertz that is a number that you see here, which means that the number of pulses hitting the sample is 800 into 10 to the power of six per second. And here what we see is number of emission events that are recorded per second 765 to see this number is fluctuating where the number at stop is not. That is because the laser gives out pulses at a constant repetition rate, whereas when you record the emission that is a more random

event, but we have kept the number of such random events as a very small fraction 1% or less, no more than 2% definitely of the number of stop events, this is required.

Otherwise, we get what is called a pileup effect and in our decay, we get a spurious fast component. So, this is what we get per second. Before acquiring a decay, it is important that we understand what are the parameters are here you see, it says, time range is 50 nanosecond. You might remember in the previous session, we had discussed TAC range. This is TAC range right here. This means that the TAC waits for 50 nanosecond for another signal to come. It also means that the full scale of our measurement in time is 50 nanoseconds.

And you see there is something called coaxial delay. You have to actually delay the start as well as talk pulses. Appropriately so that they all come in the region where we are looking. And now, to give you a little better idea, you can look at the start inputs and the stop inputs separately. Here the TCSPC source is custom because we are using a source that is not really part of the spectrometer we are using a titanium sapphire laser.

Now this threshold and all generally nowadays for commercial instruments, you do not really have to play around with this. But if you are working with an assembled instrument, you need to know what kind of threshold you have to set so that you get a good signal and it is not contaminated with too much of noise. This is start inputs and these are the stop inputs you have seen already. coaxial delay of 35 nanosecond and here one more thing that I like to draw your attention to you to his histogram size here. Histogram sizes, eight one nine two bins.

We means that in the multi-channel analyzer, there are 8192 channels. So if you it is approximately 8000. But the reason why it is 8192 is that you will see in all these measurements, it is always 2 to the power something. So you can work out two to the power x is equal to 8192. If that is a case, then what is x, and I leave that to you. But these are, whatever number you get here is always two to the power something. And that is because computer works on a binary logic. So this is something that you can change. If you change it, then the number of points will change, keeping the full scale the same.

So that will change your resolution. So once again, let me emphasize that you should not always work with 8000 points. Sometimes you might need more if possible. Sometimes it is enough. If you work with less, you need to know what kind of decay you are looking at. And you have to choose your resolution properly so that you don't end up spending too much of time recording the data, but you have data with sufficient resolution.

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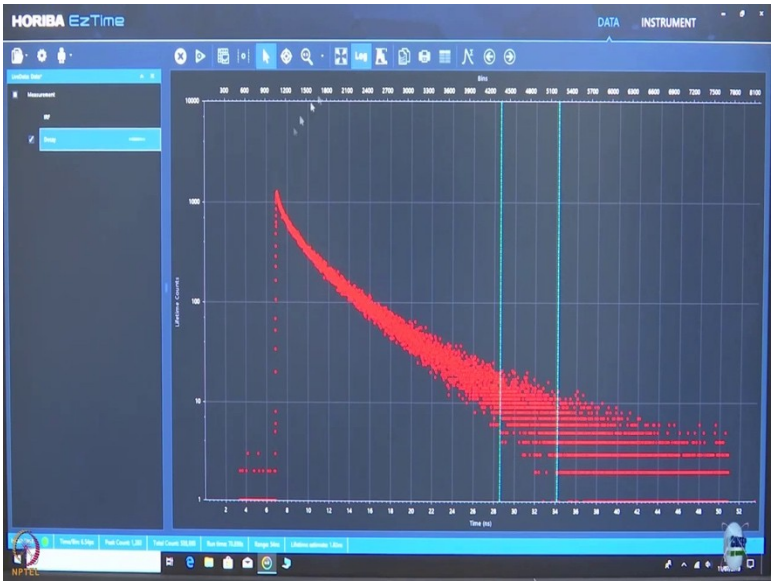

With that background we can try to record the data itself. So, you can see here we have 2 options IRF and decay. IRF means instrument response function will record it later, maybe before we do the analysis, right now, we are looking at the decay, let us start acquisition. Here the y axis is logarithmic. So you can see that the decay starts building right away. And to our eyes it looks like all the points are going up at the same time.

But that is not correct, because if you remember, we are recording about 8000 events per second. Our eye does not work that fast. Our eye cannot really tell more than 10 or 30 events per second. So, it looks like it is happening all together but actually it is not. This decay is now being built point by point. And what you see here is, this is time zero. This year is the decay and it looks like this because it is lgorithmic the advantage of having a logarithmic y axis is 2 fold.

First of all, if it is a single exponential decay, it will come up as a straight line. So if it is anything other than a straight line, you know for sure that it is not single exponential. Secondly,

another advantage of having a log scale is that you can see high counts as well as low counts together. You can see from here to here, it is about 10 from here to here is 100 and here to here is 1000. So, that is why you can actually look at the smaller decay smaller count part of the decay as well, along with the part where you have a larger count.

If I did not use a log scale, then this is the decay that we actually get Y axis now is linear. And you can see the decay is practically over by the time we have reached 38 nanoseconds, which means that the scale that we have used is perhaps good enough, but maybe there was no need to use 8000 points here. So this is how you record a decay in TCSPC. Next, we are going to record the IRF. We are not going to show you that. And we will show you a little bit about how to do data analysis.

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Now we have recorded the decay. We have recorded up to 5000 counts, but actually it is better if you record up to 10,000 counts at least. And we have zoomed in over a range of 15 – 6 that is 9 nanosecond. So you can see that decay is almost over there is a little bit more is there beyond it. This is zero time and these are the points. The next thing to do is to record an instrument response function so that we can analyze the decay that is what we will do right now.

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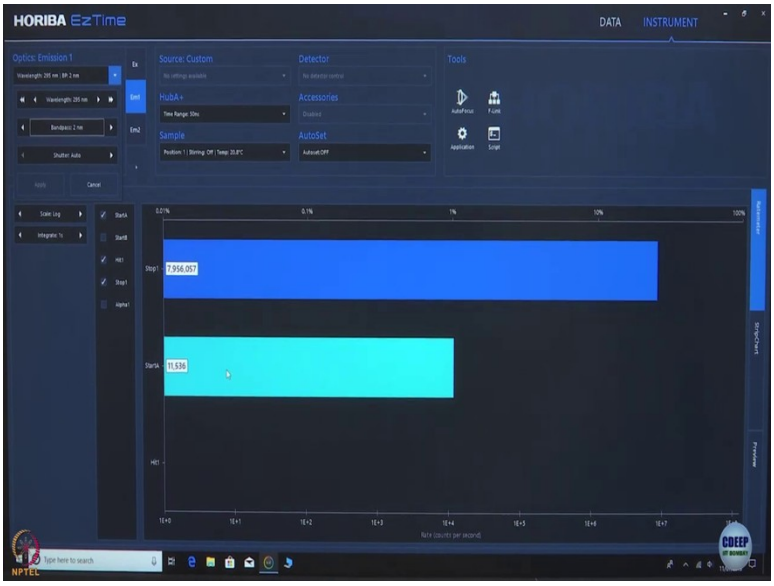

To record the instrument response function, we have replaced a sample via scatter in this case it is ludox, and we have changed the wavelength to the excitation wavelength, the earlier decay that we showed you was recorded at 350 nanometer. Now we have changed the wavelength to 295 nanometer and we are looking at scattered light and since we are looking at scattered light, you can see the counts really very high. And even though we have actually decreased the bandpass to we should reduce the bandpass a little more it was 4 nanometer.

Now we have decreased to 2 nanometer. Now we have about 11,000 counts. Remember in TCSPC, you should not have too much of counts coming out of the photomultiplier tube that gives rise to pile up effect and can also affect your detector in the long run. So, now, we go back and we record the instrument response function, this can be done in a jiffy, because first of all counts are so high and you can see this new one coming up.

There is instrument response function, which means the laser pulse as the instrument sees it, this is the plot with y axis in linear scale. This is the plot with the y axis and the log scale. So, you might see that there is a little bit of an after pulse that always comes especially if you use a very fast measurement and if you use ultra-fast lasers, but sometimes these are after pulses come as a result of poor alignment of the light into the sample chamber and that has to be taken care of by tweaking the mirrors.

So, now, we have recorded the data, your decay is here and instrument function is also there. And you might remember that we have discussed that in order to fit a decay, we have to do what is called iterate reconvolution, we have to decide a fitting model and we have to consolidate it with the instrument function that we recorded here. And we have to see how good a fit we get.

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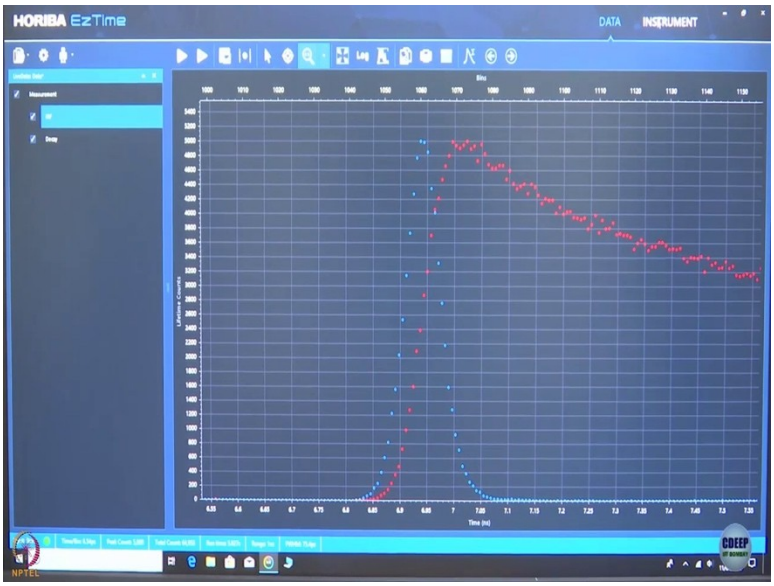

And you might remember another discussion we had, how many points we have, how many points do we have in the instrument response function, we have said that the number of points is in finite in principle, but finite in practice, because we are working at certain resolution. So, you see, each of these points is going to act as a Delta pulse and that is what we are going to use to deconvolute this data and extract the lifetimes.

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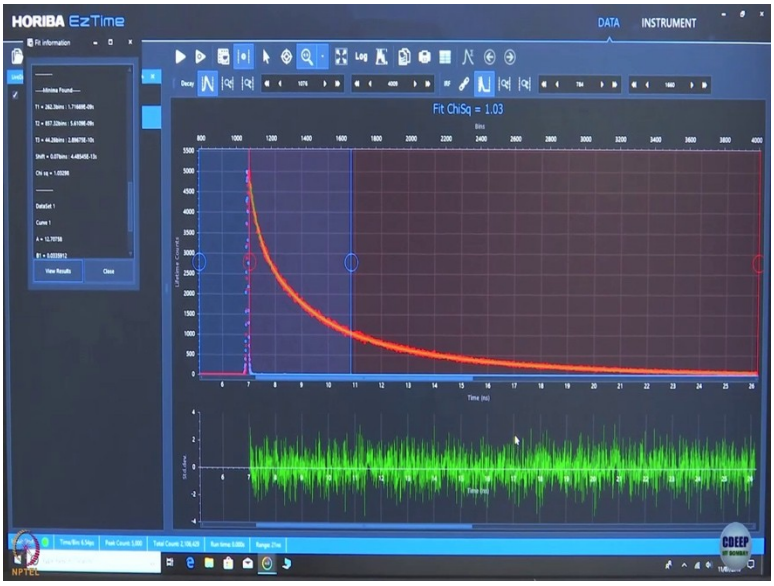

So, what you see here is the result of fitting this decay to a bi exponential function. So, this range denoted by the 2 red 2 blue lines is the range that we have set for the instrument function. So, essentially, if you remember, for getting the intensity or at each of these points in time t, we had to integrate the fitting curve multiplied by the instruments function at time t minus t dash over we had written minus zero to infinity or minus infinity to plus infinity here and for all practical purposes, you want to set a limit within which the instrument function has non-zero values.

And this is the range that we have set. But for fitting the data, the range that we have set is much larger from here, all the way up to here. And we have got the results of the fit here. It is something like the first component is 1.299 nanosecond second component is about 5 nanosecond then a shift is a measure of the difference between the peaks of the instrument function. And the decay that always happens because of something called color effect in the detector.

And the amplitudes will show up if you open this up actually there here. From here you can calculate the relative amplitudes of t1 and t2 and chi square turns out to be 1.16472. There is no need to go to so many decimal places 1.16. So, if you see a chi square of 1.16, you think it is a good fit, more or less good fit. But now, if you look at weighted residuals, I do not think we discussed weighted residuals in the last class. Maybe in the next class, we will discuss what it is.

But if you look at the weighted residuals, this gives you a measure of how what kind of fitting we have in the entire range of fit. So you see, between, say, 9 nanosecond and, 31 nanosecond, you have got a good fit. But before 9 nanosecond that short time, the fit is really bad, which means that we have to play around with this range or change the guess values that we started with and see if we can get a better fit. Now, how do you choose a range that is to be fitted to see it is up to 31 nanosecond here we could have gone further.

If you went further, then practically the data is all zero. So you have fitting zeros that will always be a good fit. But that does not and since it is a good fit, it will make your chi square look better. But it makes no sense. Because what is the point of fitting a flat line that does not really give you any idea about the time constants that you get as a result of fitting. So it is important that we covered the entire decay. We go all the way up to some point where the decay has become zero. But it is also important that we do not go any further and about the other one range of the instrument response function, it is very important that we covered the entire range where the instrument function may have nonzero values.

And to do that, it is usually better to look at it in a similar plot so that you do not miss any after pulse that is there. So do the feet once again with a little bit of different range and see whether there is any improvement or not. Now, what I have done is, first of all, I change the range, I increase the range of the instrument response function to make sure that I am not missing out on any point that actually contributes. I also made this range of the decay a little smaller. I started much later.

So you can if you can see, I am actually missing out on this much of decay, which is not good. Still, my fitting is no better than what it was if you see still the initial part is not fit very nicely, which means that perhaps my fitting model is not right. Two exponentials may not be the right function to use. Let me see what happens if I use three exponentials instead keeping the same range. Now, we have fitted to threeexponentials giving the same range.

And now you see the residuals are nicely distributed about the mean and chi square is as good as it gets, it has a value of 1.03. But still I am not satisfied with this, because you remember, we are

actually losing out on the initial part. So I would like to change the range once again and see whether they can be an improvement. shall we go now we are using a triple exponential model. And you can see the fitting has started here almost at the top and throughout the decay quite good. So, with what tells me is that bi exponential model is not all that good.

And you have to use a triple exponential model, which actually makes sense in this case, because the sample we are looking at is a protein. The emission of the tryptophan of protein is what we are monitoring here. And it is well known that tryptophan, even free tryptophan in water or some other solvent always has a triple exponential decay. Time constants we are getting are 1.7 nanosecond 5.6 nanosecond and it is 2.8 into 10 to the power of - 2 is a very small component, which actually may be believable.

What we have seen here is that, how we fit the data and while fitting a data, it is not something that gets done by itself actually it does but then it is not believable, what you need to do is well fitting data you have to spend considerable time work with a model that makes sense, because you know, instead of tri exponential model, if I use an 6 exponential model, or 5 exponential model fit might be even better, because there is something called over parameterization.

While fitting data, if you use a larger number of parameters, the fit is always better, but that may or may not make sense. So, we must use a model that makes sense for the system that we use. So the take home message here is that the fitting process might look very, very mechanical, but actually it is not. it has to be done, keeping in mind what kind of system we are looking at what it is that we expect to see, of course, that expectation might cloud our vision that danger is always there.

But you cannot do it without thinking what kind of a system you are looking at. That is what we wanted to show in this module. We get back to the class after this and we will start a discussion of the different fitting models one can use. We have already talked about single exponential and multi exponential models, but not every decay has to follow one of those rate laws. We will see what are the other situations that can arise while, fitting a data that is it for today.