Design Practice - 2
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Lecture – 37, 38, 39
Thermal Cycling for PCR

So let us discuss details of the PCR initially basically why we are carrying out thermal cycling ok.

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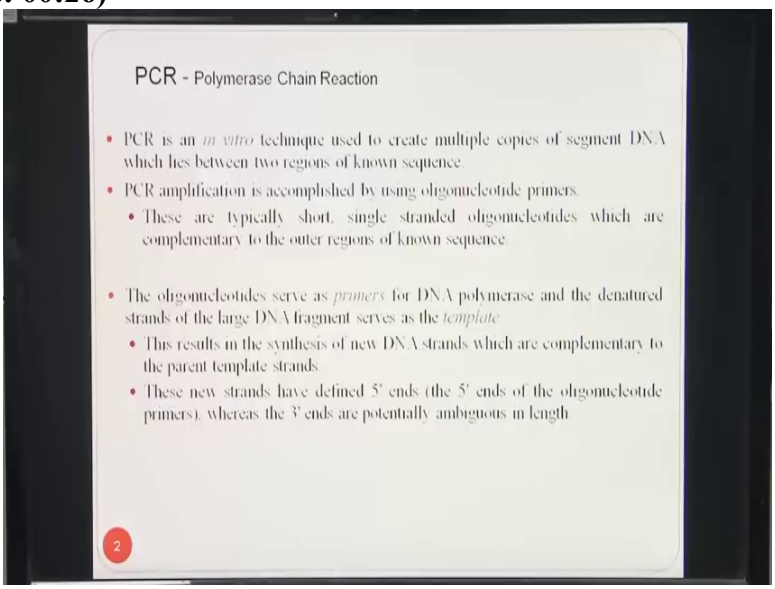

So, PCR is a polymerized chain reaction it is an in vitro technique used for creating multiple copies for of segmented DNA which lies between two regions of known sequence. PCR amplification is accomplished by using oligonucleotide primer basically. So, although these oligonucleotide serves as the primer for the DNA polymerase reaction and DNA strands for large DNA fragments serves as a templates.

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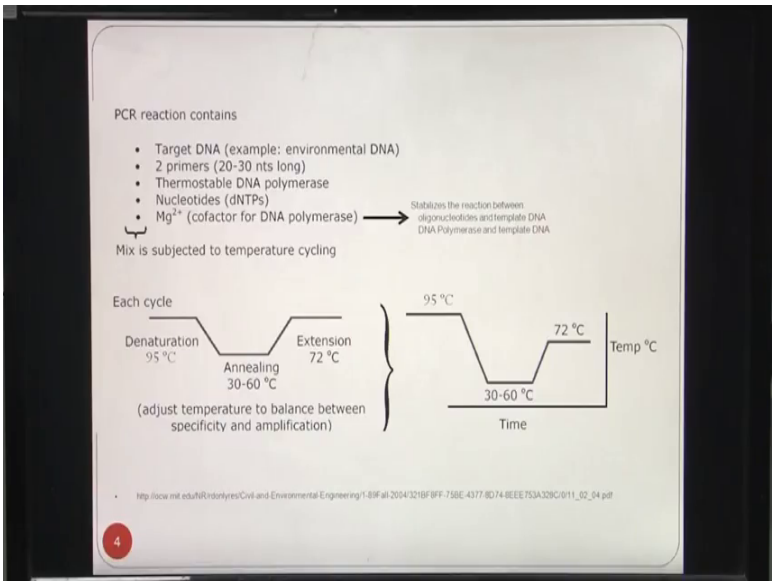

So we basically initially take a PCR mix in which the primers and different components like targeted DNA, primers thermo stable DNA polymerase nucleotides and the cofactor for DNA polymerase is basically where mixed to gather to make a PCR mixed solution. After that you carry out PCR reactions.

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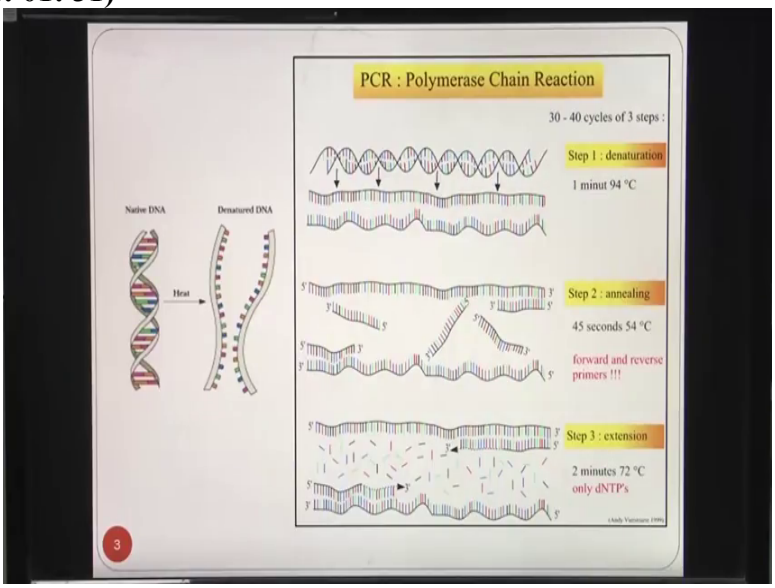

So, the basic steps, which is having like you can see here one is native DNA one is after heating we are getting denatured DNA. Denatured DNA these two strands are separated out, so this steps, happens to be at 95 degree centigrade or 94 degree centigrade after that annealing happens. So, during annealing shift we carried out annealing for 55 degree centigrade or 54 degree centigrade depending upon the requirement of required gene amplification. So the primer have 5

primes 2 3 Prime DNA strands 5 primes to 3 primes binds together with two segments which were created during earlier steps and during heating.

So, this utilizes the reverse and forward primers to make a multiple copies of a templated DNA. And then basically the extension of these carry out during which the binding happens. So only the dNTP's attached with the templated DNA.

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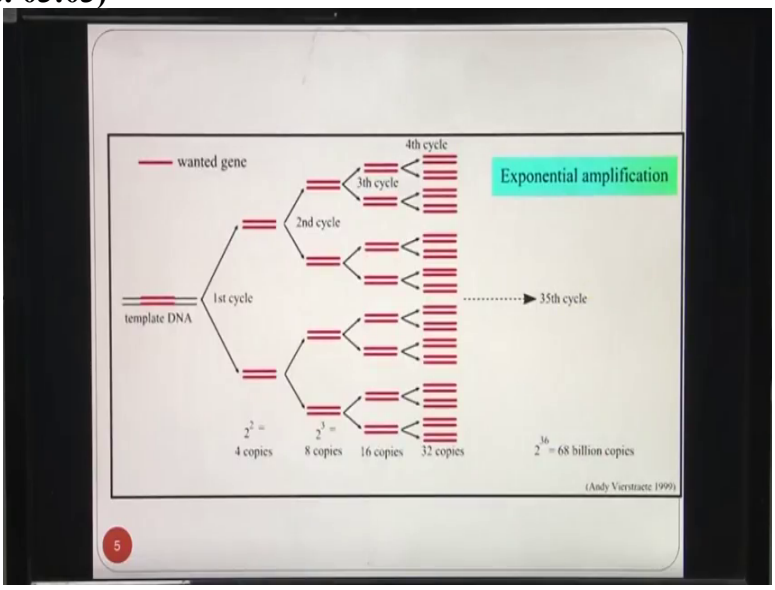

So, after that at that way in one cycle so we are having of two copies of like 2 to the power 2 that means 4 copies in a first cycle. So, in a second cycle 2 to the power 3 and likewise in 4th cycle we have 16 copies. So that way the exponential amplification takes place during the thermal cycling. So, up to the requirement how much cycle we need to be taken we can count that, at we can approximate that, can program into that manner basically how that how much cycle will be required for that at this purpose. So, it will depend upon this situation by situation of gene type etc.

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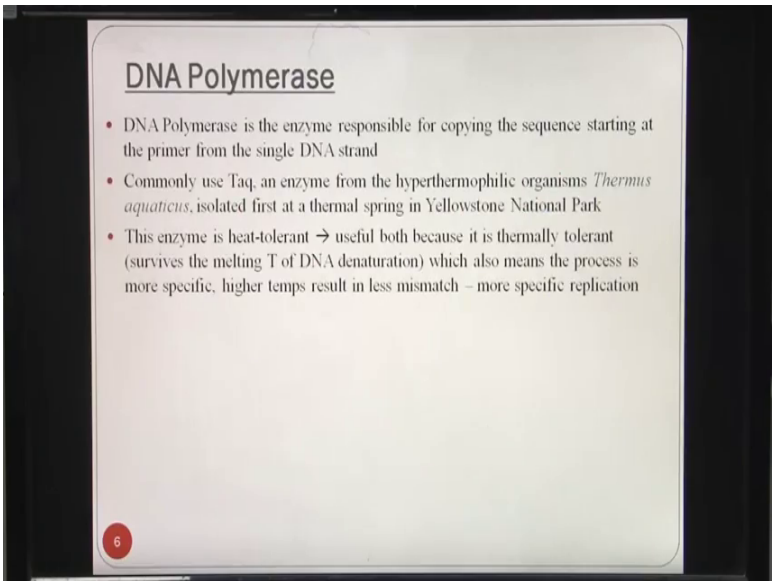

DNA polymerase is; let as talk about DNA polymerase as DNA polymer is an enzyme responsible for or copying the sequence starting at the primer from the single DNA strands basically. So commonly used; we used of there are different kind of polymerase which like Taq, generally we use is Taq polymerase and an enzyme from hypermophilic which is basically Thermus aqua Thermus Aquaticus isolated from thermal springing Yellowstone that is basically a created at National Park.

So, this enzyme is heat tolerant basically that is why we are using it also we add for the heat toleration we add this magnesium cofactor for DNA polymerase basically for the stabilization of these enzymes and also oligonucleotides like dNTP's so that they did not get denatured so to carry out the polymerase reaction.

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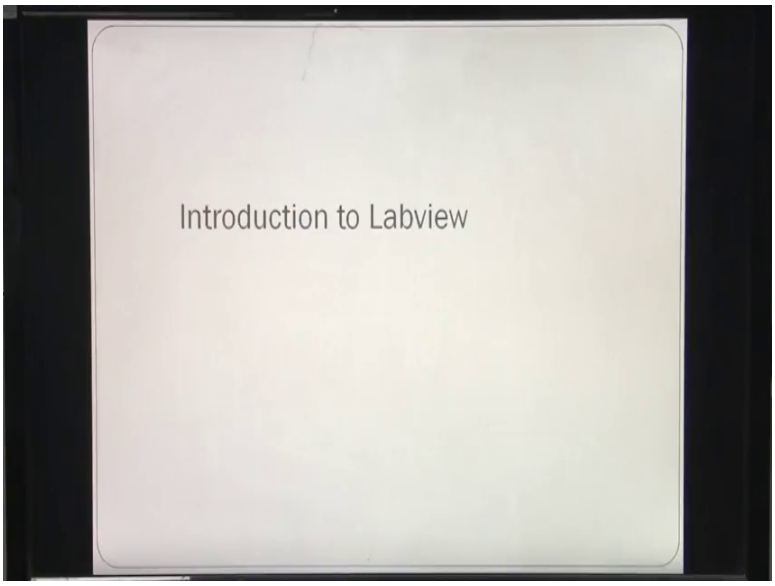

So, now we will focus on; so, as per we discussed about thermal cycling we have to carry out to perform PCR reactions. So here an instrument it is while national instrument which basically gives an opportunity to program graphically encoded form so that we can maintain temperatures like we have discussed 95, 55 and 72. So, now we will have some small introduction about the LabVIEW. How LabVIEW works on the; like information of how the information flow into the program, so we will discuss about that.

So, there are various different blocks interfaces like for loop and while loop etc which is uses in word language like C, Java etc. So, it is far different from that, it uses graphical language basically. So, the program is called VI that means virtual instrumentation. So, whatever the program is made in the LabVIEW you will be created in the Vi form. So we call it Vi virtual into to instrumentation so that means these are the instructions which is given in a program to perform certain reactions like suppose we want to monitor temperature of a particular thing.

So, we need a Thermocouple and we also need hardware could be which could be we able to sense those temperature and to digitise those sensed data and to give to the National Instruments and then it will come to the as per the program instructions. So, let us have a look at brief introduction on LabVIEW.

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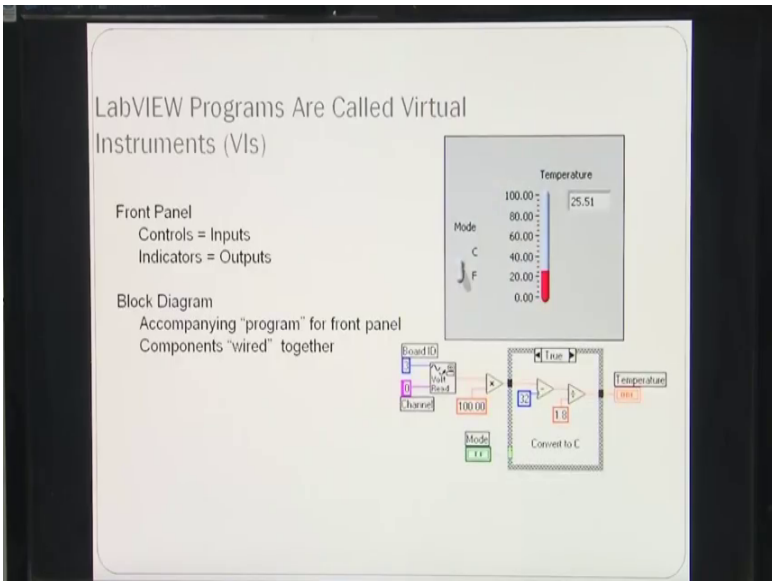

So, starting to the introduction of LabVIEW, LabVIEW program are called virtual instruments basically. So it comprises front panel and block diagram basically. These two components are put into the particular software. So, here you can see she so like here you can see front panel, this one is the block diagram. So, in which basically formation when we enter the value for some execution takes place. So, the information takes place like the; the year the program is made for convert convergence to C. So, we will give live temperature into Fahrenheit which will convert into centigrade.

So, here basically loop, while loop is used and then basically some multiplication factors are there. So depending on those multiplication factor it calculates the degree, centigrade, temperature and it can be she shown or displayed on the front panel basically. So, the block diagram still gives you the details of the particular program.

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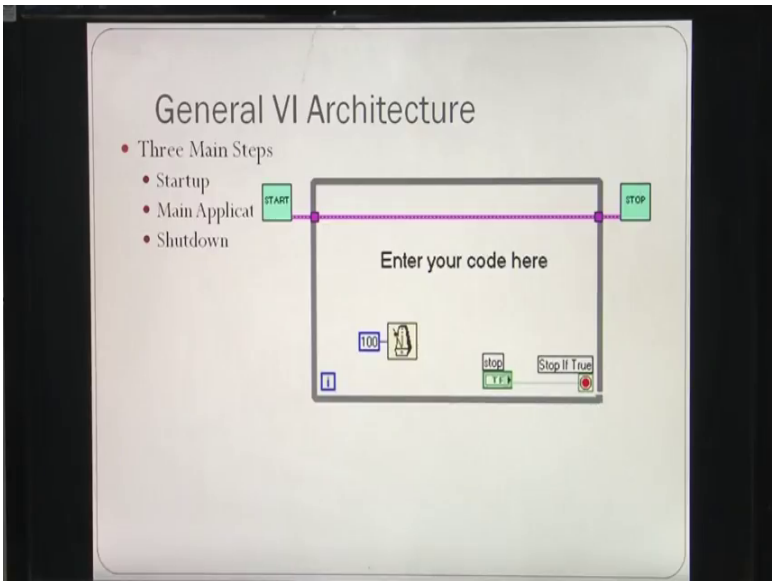

So, general architecture of the; the these are 3 main steps like we can and I have to create like from which from the point it from where we are starting and we have a while loop or a case structure or a for loop etc. In which we put graphical instructions which already defined in the basically LabVIEW. We call it we call those VI's and to create our program. So, here we called it like number, here we call like stop. Here we called VI's of stop if condition is true.

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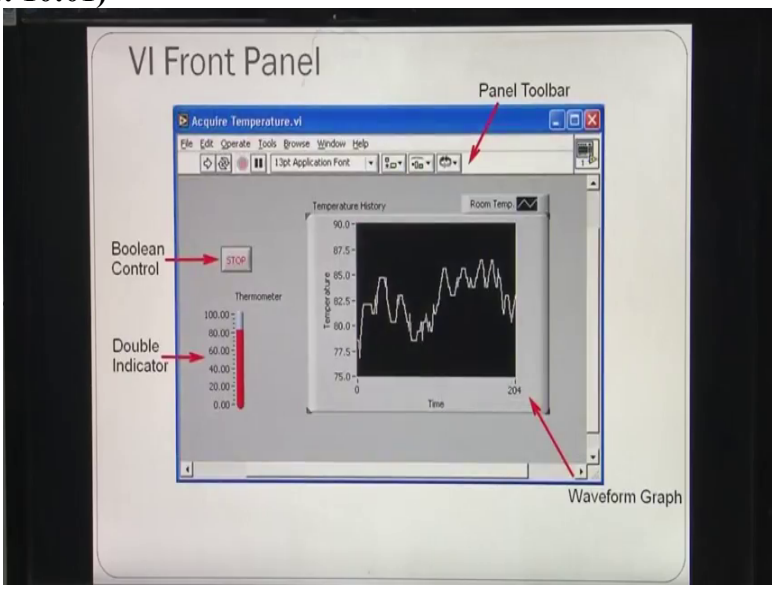

So, this is VI's front panel, so front panel is like it is like stop button is there, either it will be stop or will be start so it is operates like Boolean control. And this is double indicator, double indicator means it is basically indicating the temperature which is shown in the history and the graph like waveform graph so this is front panel.

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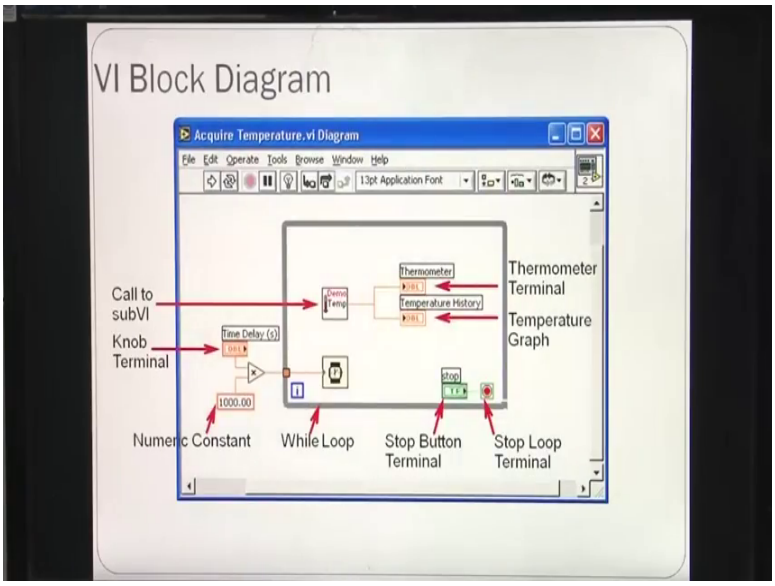

Open this is block diagram of that particular thing like I already talked about we have called this Sub VI, we have called sub VI which already adjust in the LabVIEW. Here the note the thermal has been specified which we have to stop or start. Here the numerical constant like suppose we convert to Fahrenheit to degree centigrade so which value as to be converted Fahrenheit to centigrade. So that value we have to enter it and then start and this will give you the output. So, that way; here the thermometer terminal is called and this is thermo temperature graph will be plotted over there.

Which is basically this shown on the front panel then after the calculation we can stop. Stop button is also provided here so that is shown here in the block diagram. So these are basically main component of particular LabView program. So one is again I am repeating one is front panel and other is block diagram.

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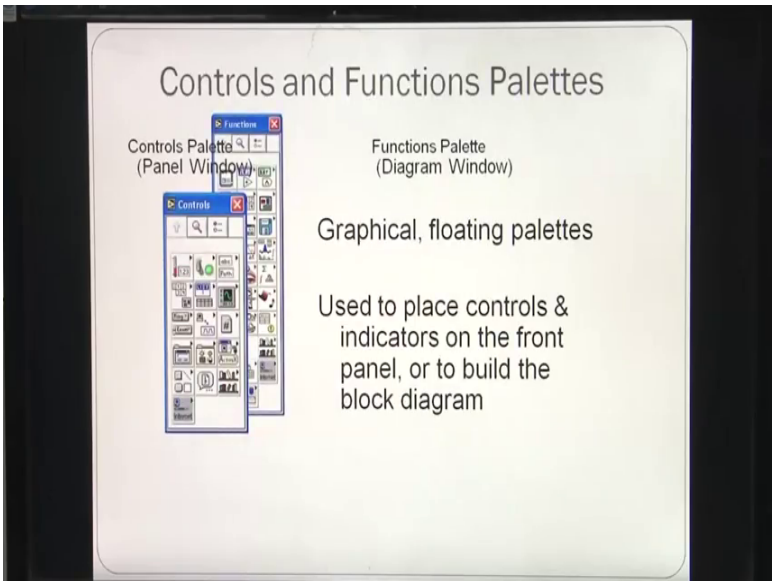

So, these are control functions palettes basically; I will be showing to you in the real software how, from where which place you can find these functions and controls. These are different controls are available in the LabVIEW so this can be a floating palettes these can be a floating palettes, graphical palettes used for different purposes like place control and indication to build a particular program which will be in form of block diagram.

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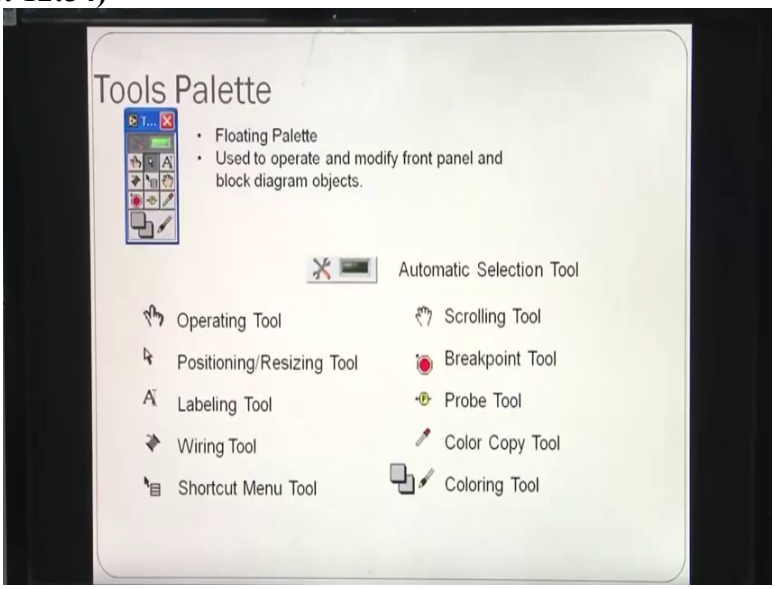

So, these are tools palettes, so there are different types of tool palettes I already discussed about that. So, these are the symbols which is show here like operating tool, positioning, resizing tool, levelling tool, wiring tools, shortcut menu, scrolling tool, breakpoint tool, probe tool, colour copy tool and colouring tool ok.

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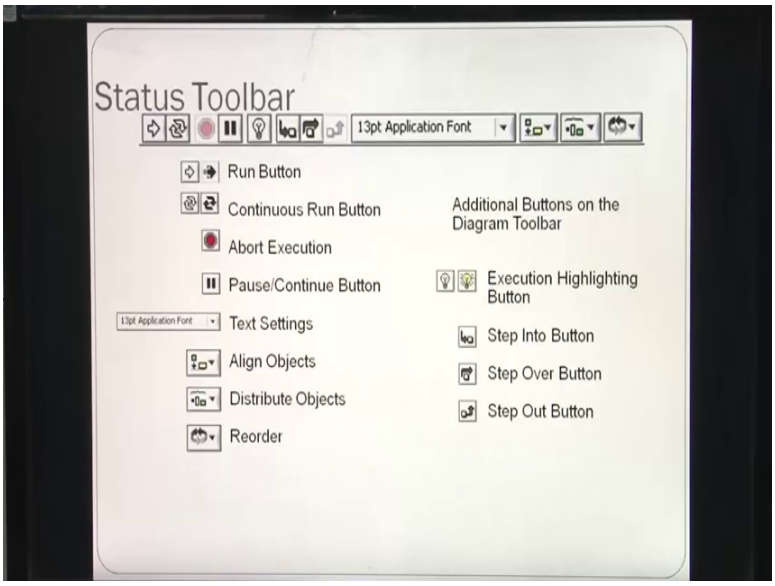

So, this is status tool bar especially it carries Run button, continuous Run button, and then abort basically abort or execution of particular program like if you want pause the program and then can we can pause this button and we can switch on this button pause. And these are a line object, these are distributed object and this is the recorder. If suppose if you want to record or add something during the cycle so that can also be done with this particular button basically this symbol which is available in status toolbar. Likewise these are additional symbols which we available with different kinds of work this.

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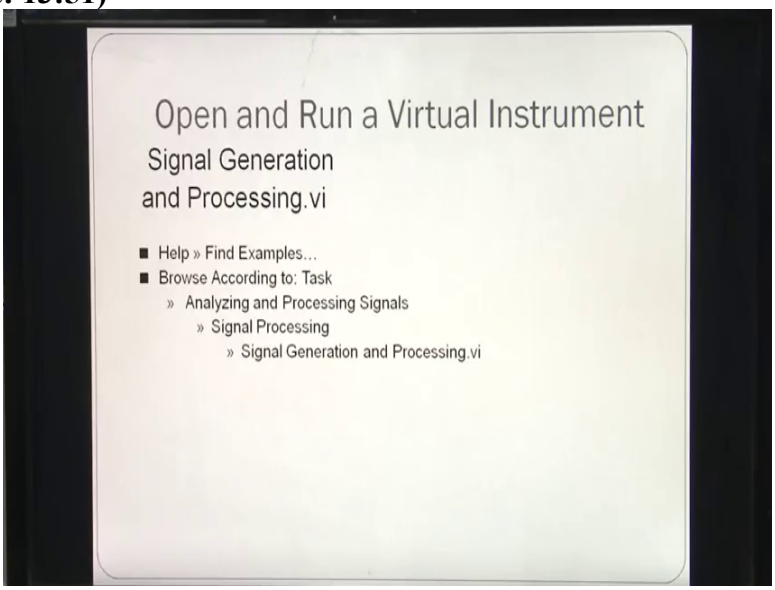

So, open and run the virtual environment, so we will basically firstly we will open and run the virtual instrument program.

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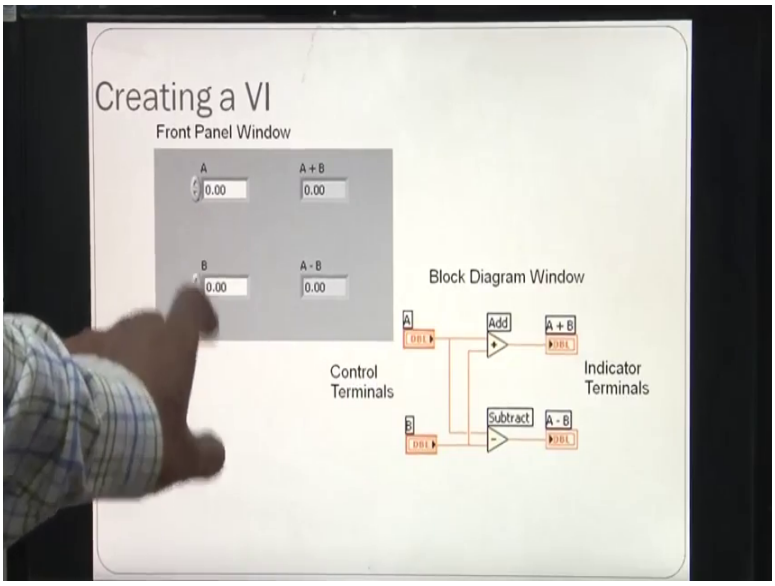

So, let us move to or let us discuss about single program like front panel have a A value and B value and so now the operation has to be done like a + b and a - b. So, whatever the value entered in the A block and B Block so that way it will add the value or it will subtract value and which will be we are basically; which will basically will be done after execution of the program. So, let us see the block diagram of this. So, block diagram of like this will be like we have used a multi subtraction symbol.

And then add signal and we have provided this; for providing the value we have a different sub VI's, so that way we can have those input it call input tabs those tabs there we can and enter the values. And to execute the program it will give an output of of a + b and a - b. And that way it is a very simple program basically. To show you how these to panels basically works.

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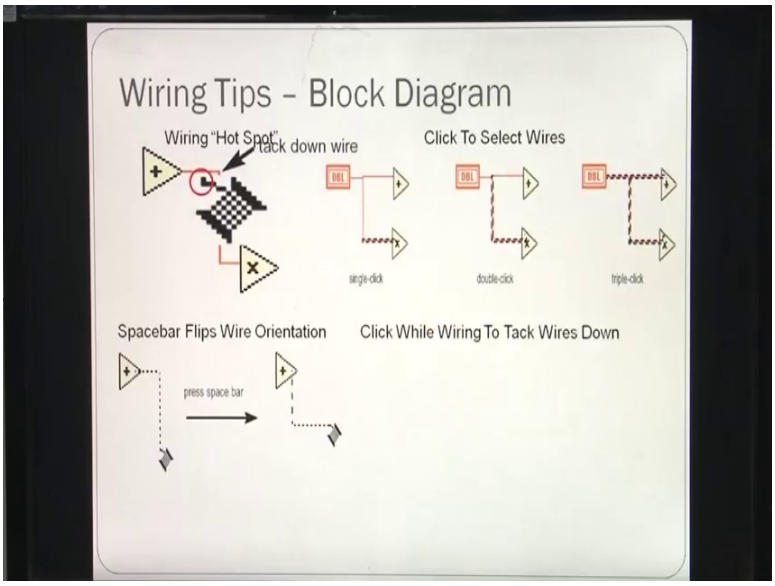

Here the wiring tips for the block diagram like we as you can see here in the block diagram, like this tab is basically symbol is attached with this particular symbol, so, how the information will go to this place. So, the wire is attached to these two symbols. So, that whatever the values that entered year is has to be transferred to the next operator which is basically carrying out addition, so that is why this these wires are connected.

So in the different languages like C and Java etc, some parameters which carry the information, but here in the graphical language in graphical language these wires are basically forwarding the language from One VI to another VI or one symbol to the another symbol ok.

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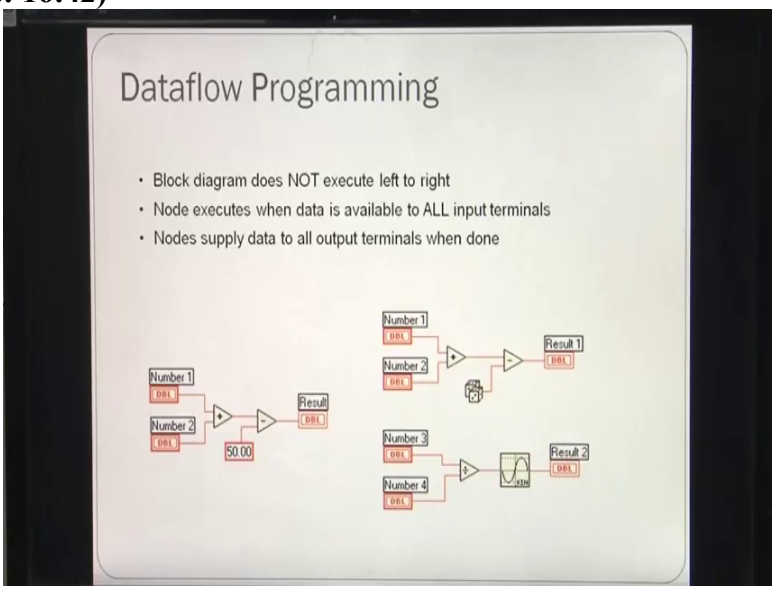

So, these are already talked about it these are that are Pro program basically how the data will flow, I already discussed about that, if we enter a number 1, number 2 it will basically after a

some operation it will give you some result it will be displayed on Front panel. Likewise these three see this three programs are there, so that you can see.

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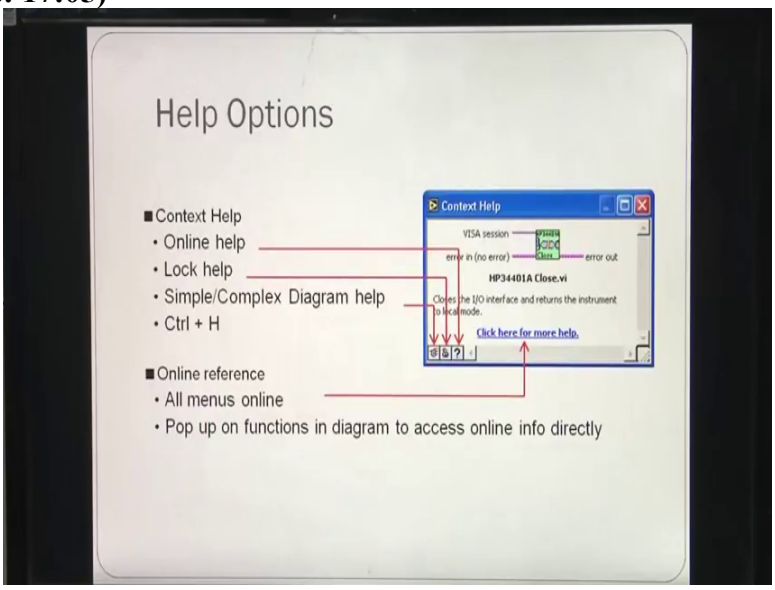

So, there are if you have a problems and you are not able to understand what it lost symbol it stands for or you can go through the for help for so that is available in the software.

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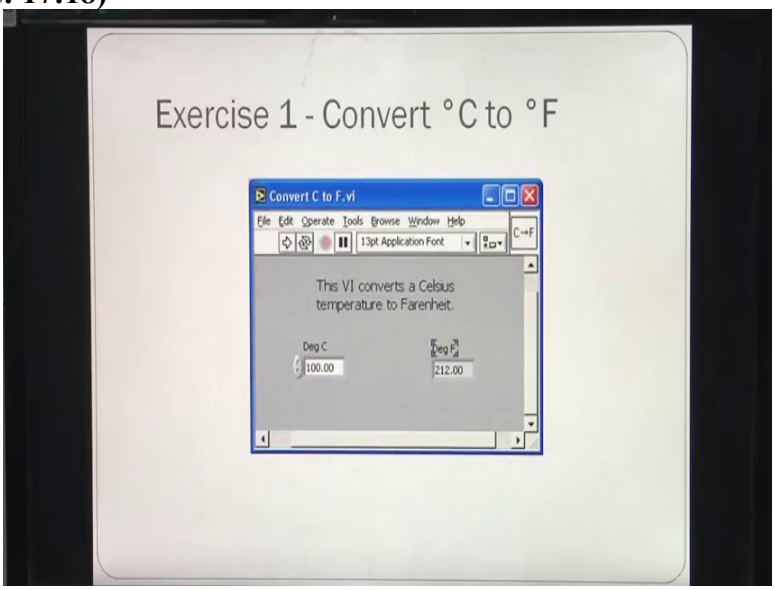

So, this is exercise 1 basically where the degree centigrade has to be converted in the; the this VI converts convert Celsius temperature to Fahrenheit basically.

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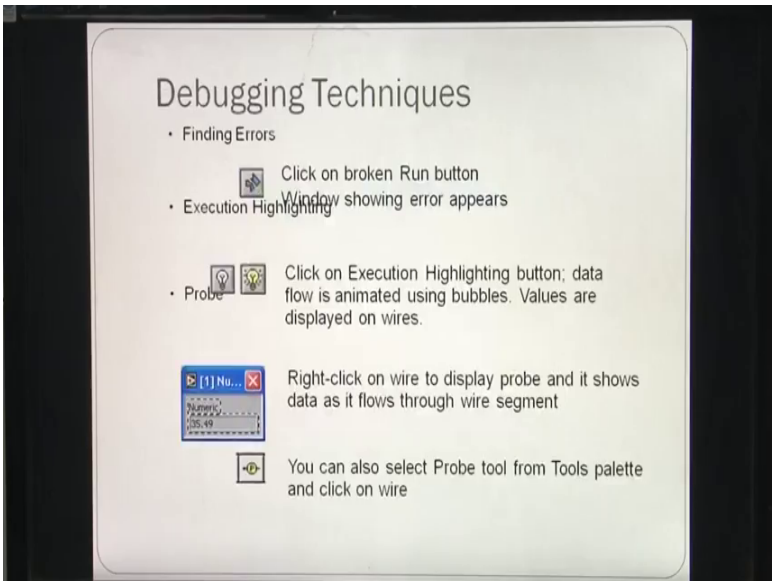

So, these are debugging techniques; debugging techniques we the before the execution of the program in actual setup. We would like to have a look how the program will behave. How the information behave from one block to the another block basically that we have created for the VI's. So, then can be assure about those things ok when the information is flowing in a right manner all the VI's which were basically called in a creation of a particular program are executing properly. So for the debugging we carry out this step.

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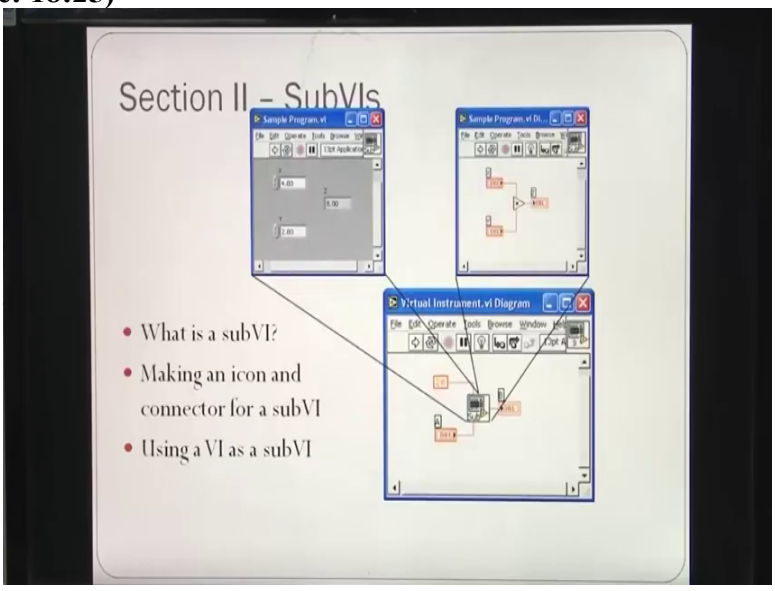

And these are they sub VI's basically, like sub VI's are single VI which is called in main VI's file basically in which we are getting; we are programming and these sub VI's are called basically main programs. So they will work according to the, their programming.

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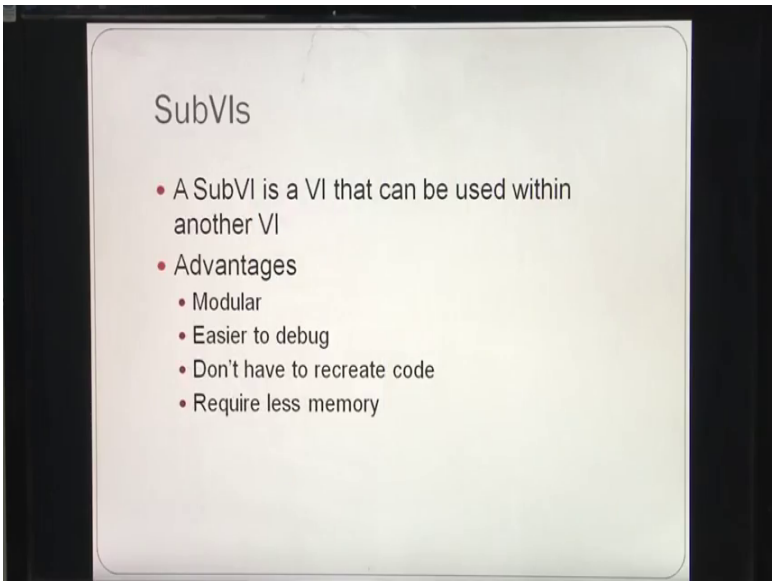

This sub VI's that can be within the another VI, I have already discussed. These are basically the advantages of the sub VI's, this is modular one that is easier to debug basically. If some correct program is there where we will call it into the main program and this program is already correct. So, it will be easier to debug and that particular point programme that will be error free. We do not have to recreate the code basically requires less memory also.

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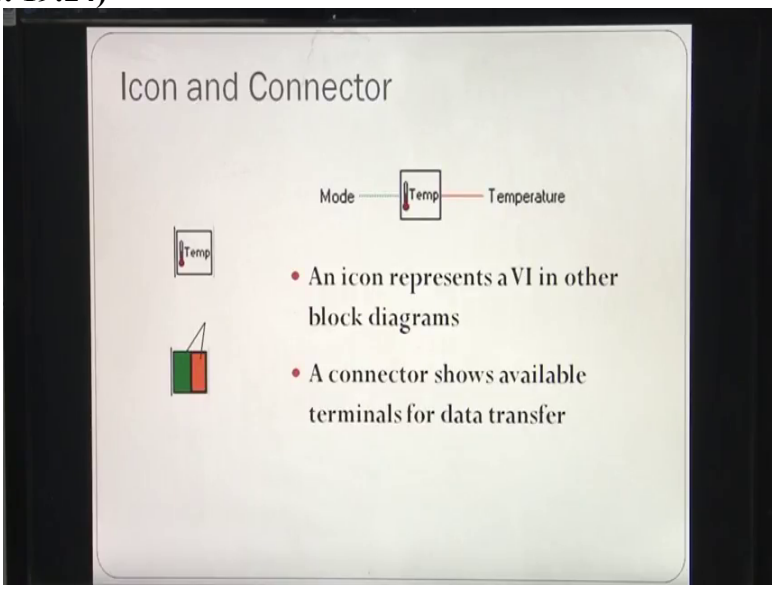

These are icon and connectors basically here we can and see those connections. Connections are made from form of difference wires like green and some orange tab so you can see mode and temperature. So, this connection basically made over there in a different form.

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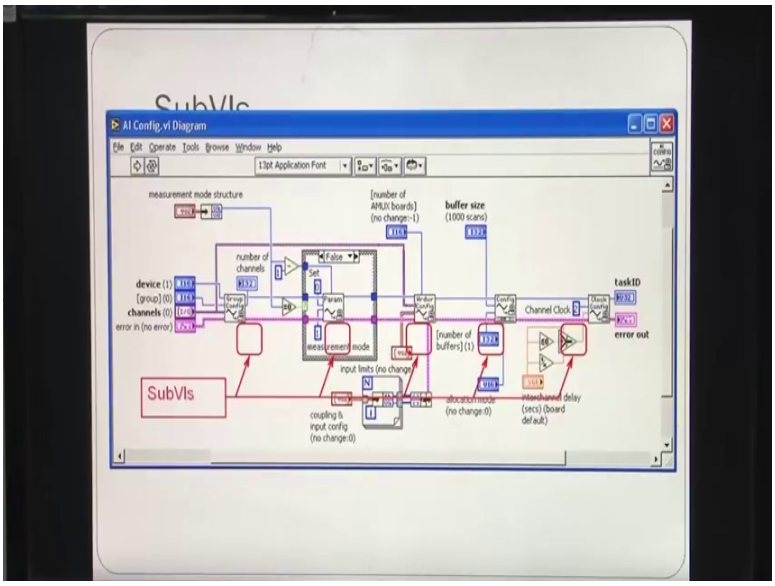

Here the; you can see here complicated program so that utilizes different VI's basically. So, like one you can see here one VI is called add and different VI is called it, it here different VI it is called; and those sub VI's are basically assembling together in a sequential manner and making and giving you a complete program for a particular to complete; to complete a particular task so in that way sub VI's are used.

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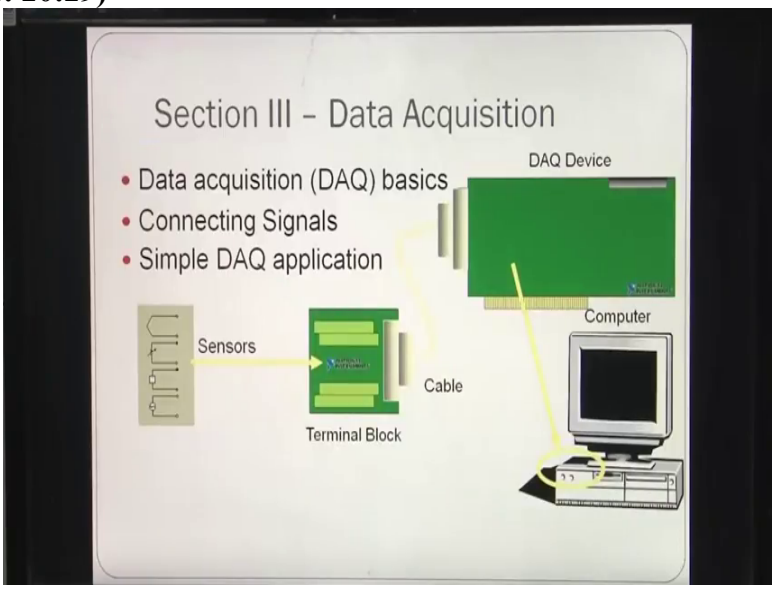

So, these are data acquisition systems basically how we can and data acquisition can be taken place. So, we have hard hardware components also. In which basically we have a those DAQ devices data acquisition devices and connecting Signals and simple data applications which is inbuilt with the sensors those acquisition devices send signal to the sensors. And those sensors

particularly basically send signal to those DAQ cards. So those day chords basically supply information to the LabVIEW the interface.

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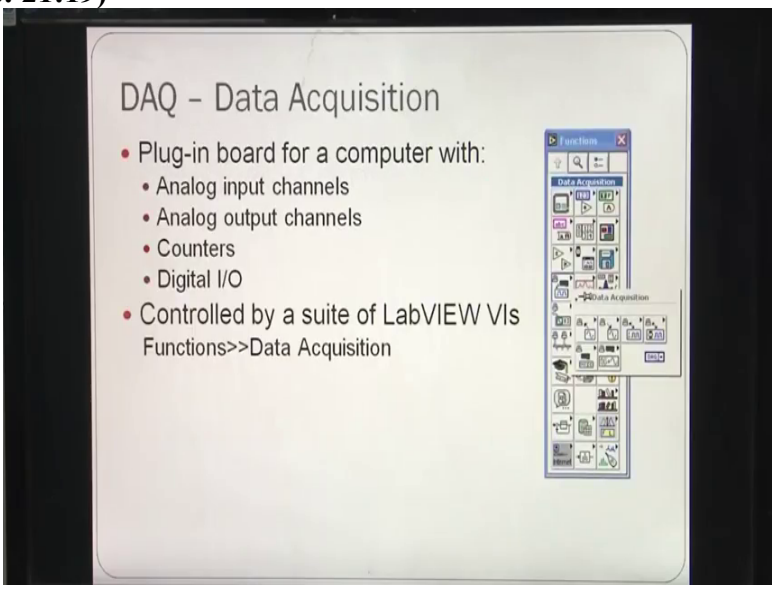

So, these are basically data acquisition systems in which plug-in board for a computer with analogue input channels and analogue output channels. And so these are; and counters, digital input/output.

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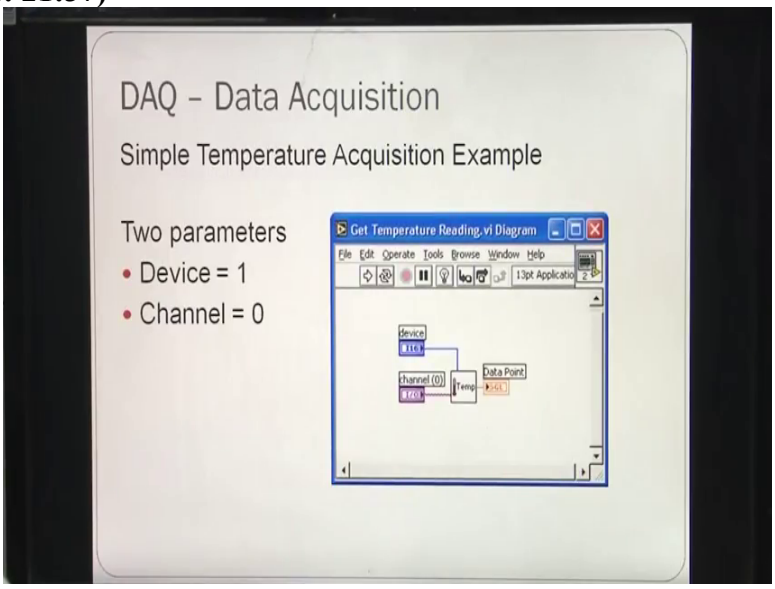

This like simple temperature acquisition example is this like two parameters I have device 1 or channel zero ok. So, device 1 which is called here 116 which will be kind of Thermocouple will be measure the and in which channel basically this Thermocouple is mounted so channel address is there. So there is channel address is there so that is way we are clear, we have created this data acquisition. So, after the sensing data the data will be displayed on the front panel.

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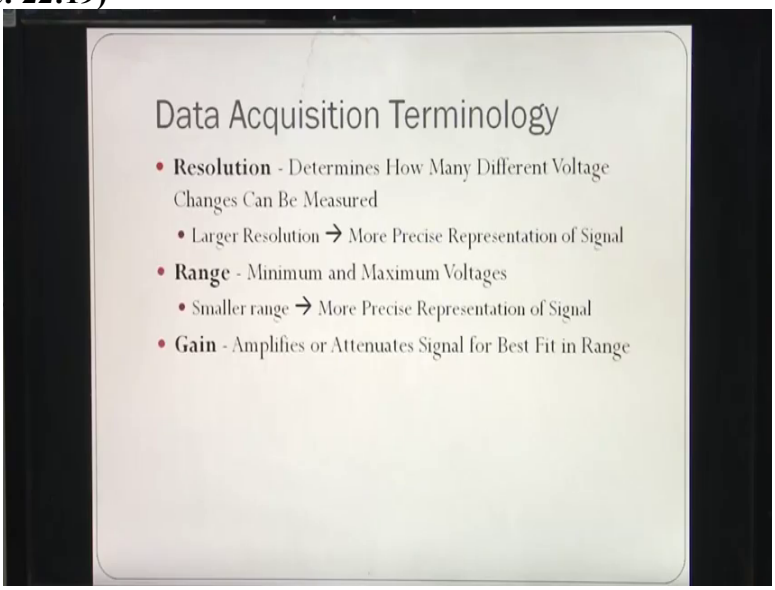

So, this is data acquisition terminology like resolution range and gain. Basically these are important it characters for data acquisition DAQ cards.

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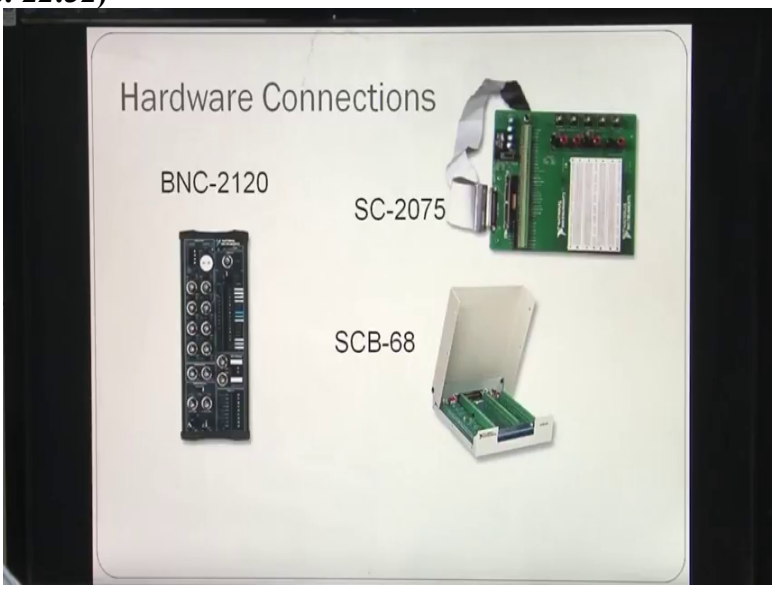

So, these are small like these are small some type of DAQ cards which is used for different application.

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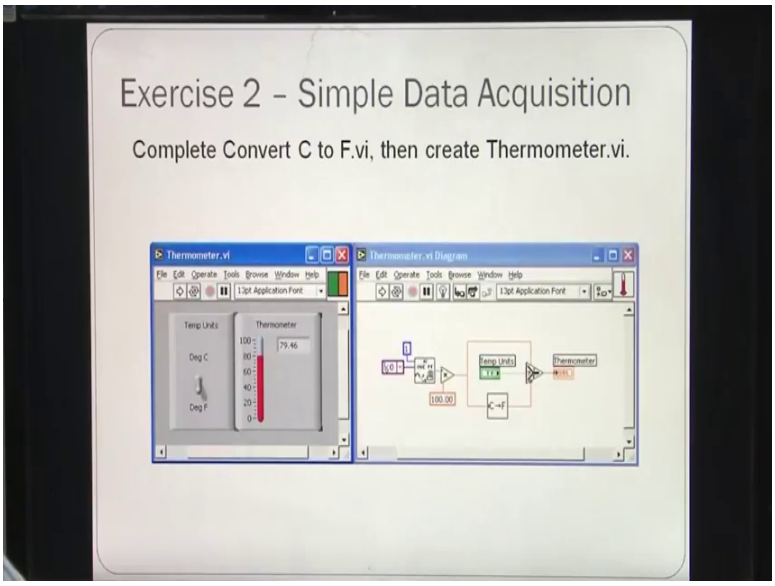

Ok so again this is showing basically in exercise 2, here you can see here front panel is there block diagram is there. So, the block diagram again it is converting the degree centigrade to Fahrenheit or Fahrenheit to degree centigrade ok degree centigrade to Fahrenheit. So here also you are applying you are supplying this 100 degree Celsius that is can be converted into to Fahrenheit. And that is getting displayed on a thermometer basically on a front panel which is this one.

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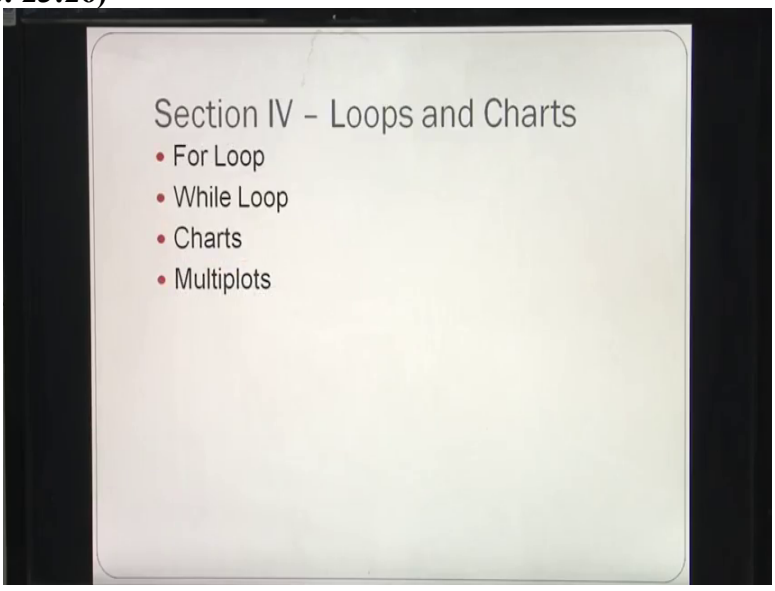

So, let us discuss about loops and charts. So, different loops and charts are used in LabVIEW program for loop, while loop and even charts and multi plots.

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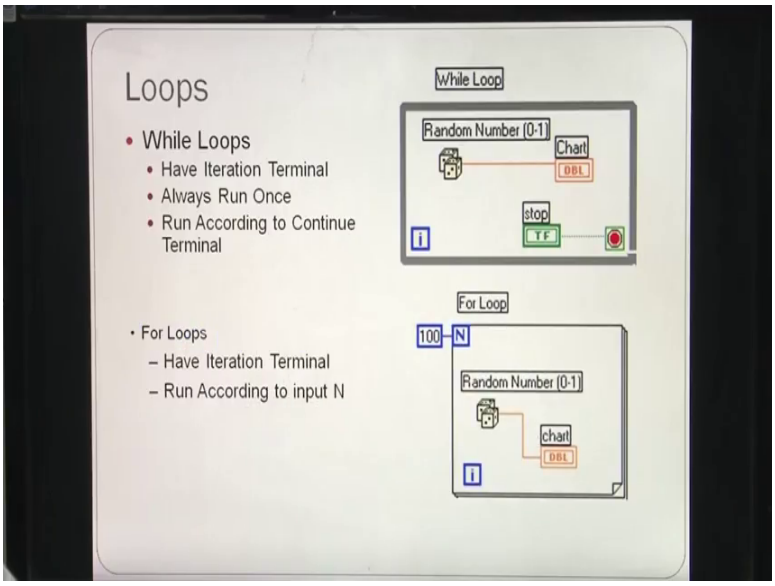

Loops are basically having iteration terminal like while loop basically having iteration terminal always runs once, run according to the continue terminal. While the for loop as iteration terminal run according to the input value whatever value you will provide like you want to run a program supposed 10 times, so you can enter the value n= 10, so that way the program will run for a 10 cycle only or 10 points only. Whatever is required in the program so that way main it basically differentiates while and for loop.

Like here you can see you have supplied those charts whatever the value you are creating can be displayed on the chart and here also we have provided like 100 value, you are not provided here the value because while loop works in such a manner that when you switch on and it will run thoroughly until unless you stop it. While the for the for loop basically will run up to that particular cycle or a point, which you will specify here. So these are important looks on which whole LabVIEW program works.

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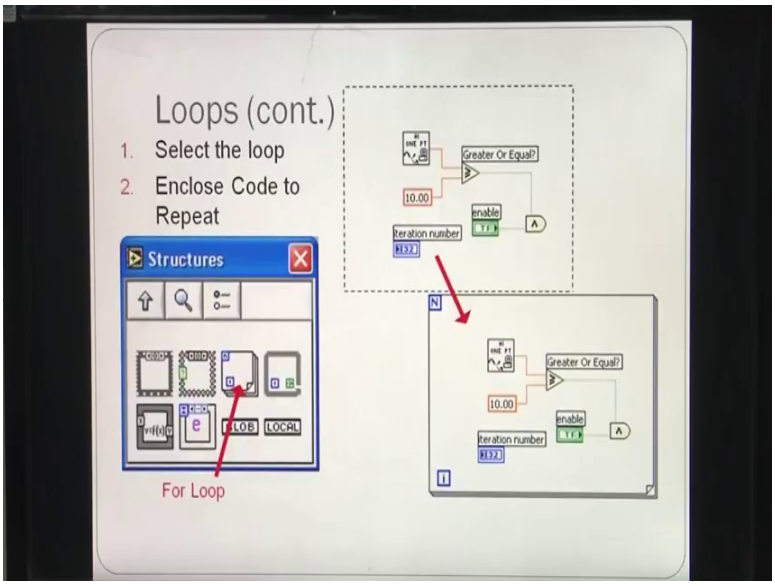

These are loop structures so this select like these are different loop basically show near. Here you can see this symbol basically source for loop. For loop basically; if for loop is applied so, here that way you can call here like this so if condition is given greater or equal. So, whatever number you supplied and number is there initially. If it is greater than it will so to the front panel or basically iteration take place depending upon the coding instructions.

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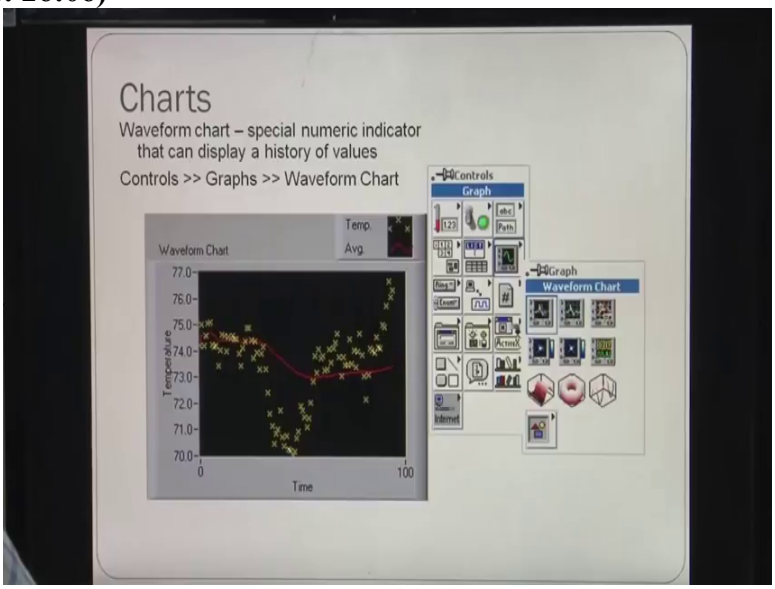

So, these are charts basically whatever value comes as output or can be plotted over a chart basically in various parent form of different parameters in which you want to plot. So, these are symbols for plotting the graphs basically. These can be coded these are the small VI's which already exist into the LabVIEW basically which you call in the main program which will create those charts. So, that way which type of chat you want it in that way you can modify the chart

according to your requirement 3 dimensional view study you want to carry out or 2 dimensional study you want to carry out. So you can use those different charts for a different purpose

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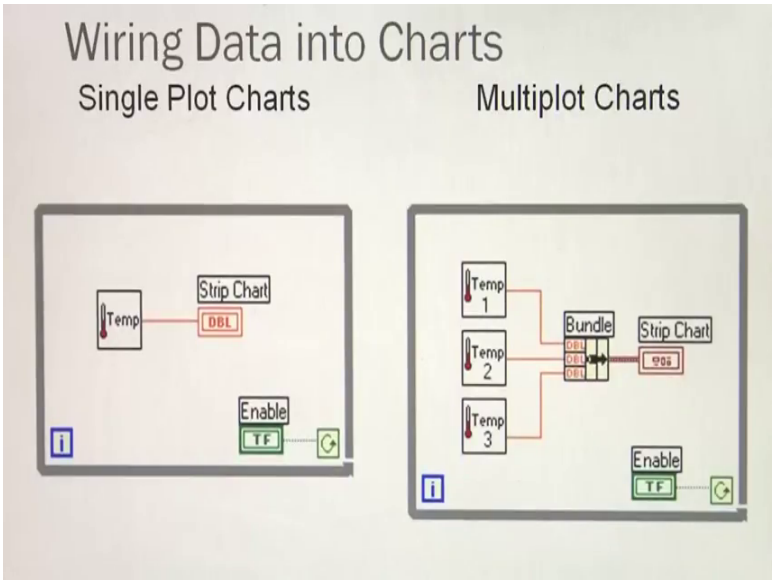

These are wiring data into charts like single plot charts and this is Multiplot chart. So, if you see here we have only a single string over there. So, temperature directly, see what ever temperature is there is directly we are displayed over there our in the form of in the front panel. While here you are three temperature you have a one done. So, suppose you want to create a charts for a every temperature so that way you can have multiple charts. So, this is for creating those multiple charge you have a different symbol.

While for a strip for only one chat, for single chat you have a different symbol so that way you can have those multiple symbol multiple charts.

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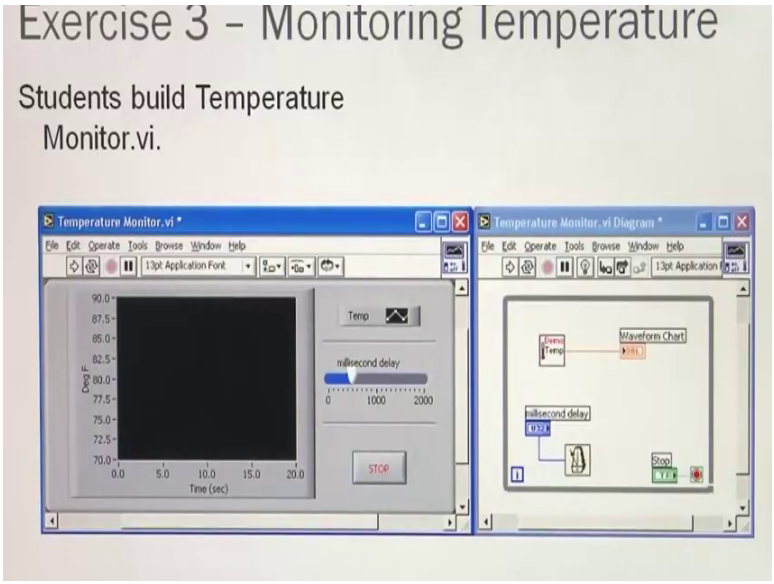

And this is like monitoring the temperature ok so again this is the same program basically which we had discussed about.

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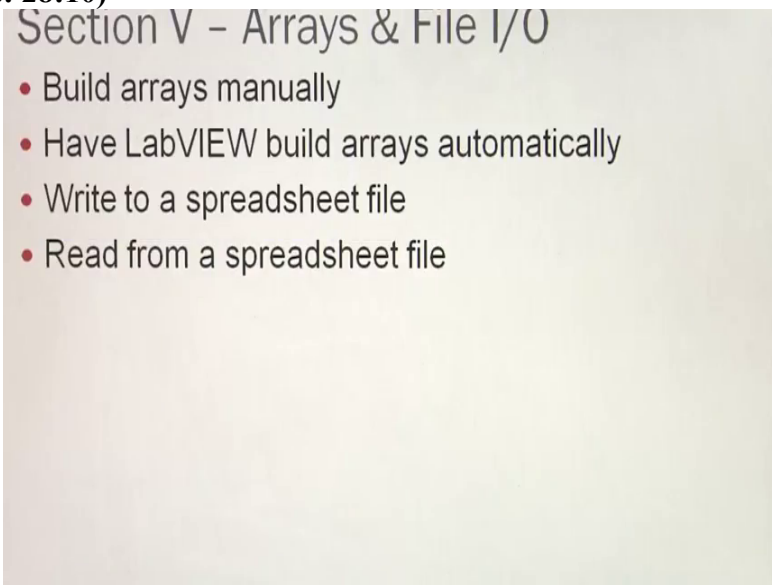

So, this are the array and file input output. So sometimes what happens like values are created different values the number of values are much more so we wanted to create a array of that basically. So, to make an array like 3 by 6 or 6 by 3 we are 10 by 15, 15 by 10 and depending upon the requirement. So, we can create those arrays automatically as the requirement of the operation.

And those created array values can be write down basically into the spreadsheet, which spreadsheet can opened in Excel are some WordPad. So, from that particular Excel or the Excel

sheet or the Wordpad we can read those information how in which column in which row which value is there so that we can see those values.

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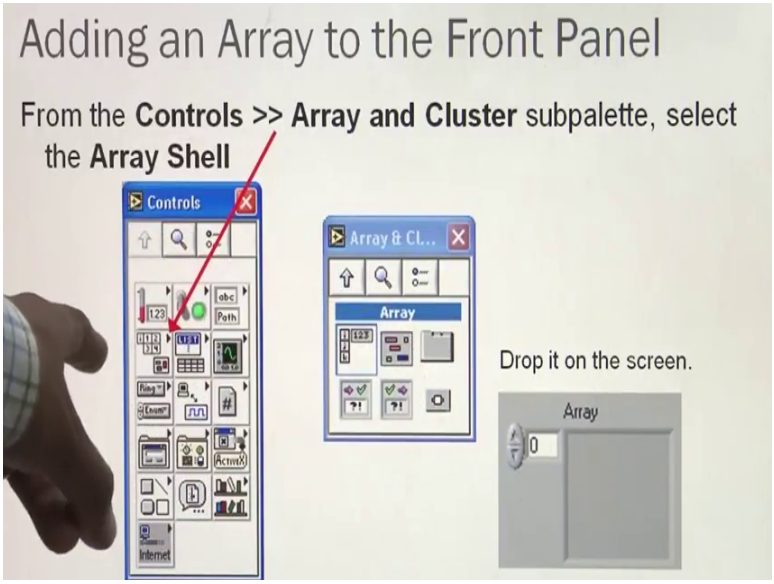

And these are basically adding array into the panel front panel basically. So, here you can have a different symbol basically. Here you can see a different symbol to control those arrays like you can see here you like you want to have a 3 by 6 or 6 by 3 that you can define here. So, these are basically in the LabVIEW on the screen like; when you open the lab view screen you will have a control option there which array and there are sub palettes available.

By which we create those array cells. Here you can see these are the; this is array and cluster. Like this way you have a drop you can drop on this particular symbol ok and front panel. So that we can create a an array automatically but we have to provide how much row and column has to be there. So, that we can have and array and cluster. This is for array and this for cluster basically.

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Adding an Array (cont.)

- Place data object into shell (e.g. digital control).