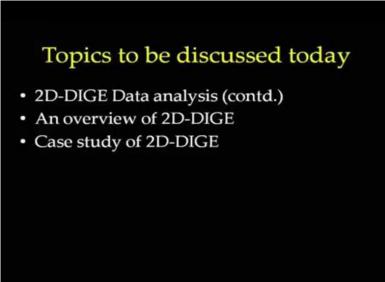
Proteins and Gel-Based Proteomics Professor Sanjeeva Srivastava Department of Biosciences and Bioengineering Indian Institute of Technology, Bombay Mod 05 Lecture Number 19

(Refer Slide Time 00:14)



Professor: Doctor Srinivas from GE Healthcare, who is going to talk to us about DIGE technology and give us a demonstration on software to perform DIGE gel analysis.

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Professor - expert conversation starts

Expert: The next one is Differential in Gel Analysis shortly we call as DIA

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Expert: In this you can see we can create a new project, a new DIA here like there is an option, create workspace.

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Expert: From here we can .it will take you to where we already saved our gels in our database Now from the database, we can select any particular project and from there.

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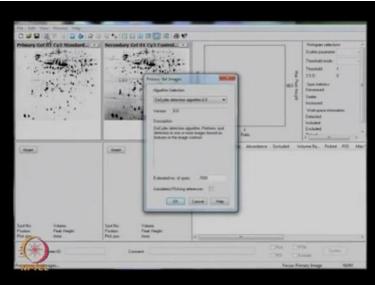
Expert: I am selecting Gel No 1, as we save this one

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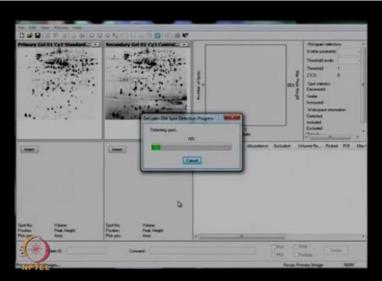
Expert: Now you can see these gels where we have uploaded. So now after uploading here, you can process these gels, then

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Expert: during process you have to give some number. This is some threshold which you are giving here actually. This will be 2000 so that it will take care of background issues also

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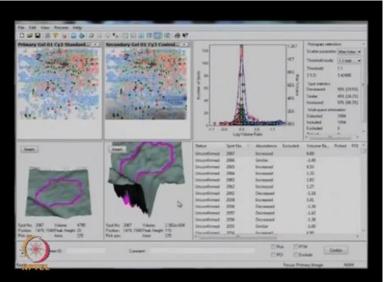
Expert: Then basically in DeCyder, co-detection will happen. But I would like to explain you some more about what is co-detection? This co-detection is, uses the information of all 3 channels and will create

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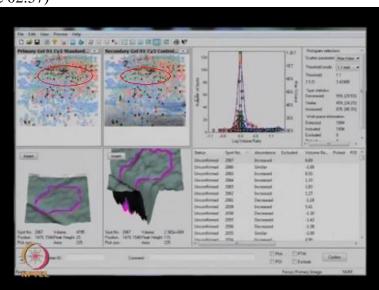


Expert: a geometrically identical

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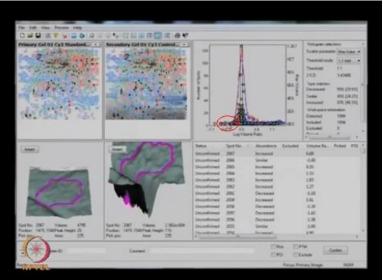
Expert: spot boundary for a spot across all the channels. That means, there are 3 channels; Cy2, Cy3, Cy5. Out of these 3 channels, in Cy2 image, it creates a particular volume and the same area can be applicable for the remaining two gels also This is way it works. In this way quantitative and qualitative results are much more accurate than with a single detection In DIA, each image is co-detected with its internal control producing 2 images pairs. The ratio of standard sample is calculated further Or the ratio of standard sample is calculated for each protein in each image.



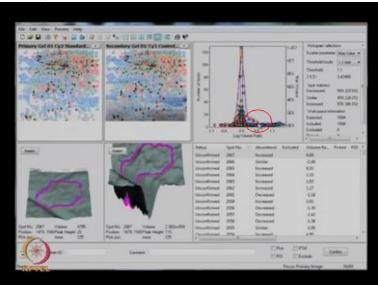
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Expert: So as we see here, these are all the number of spots it has been detected and which

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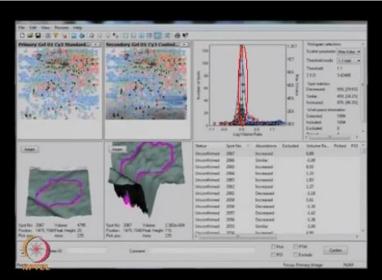
Expert: there are, you can see some red color spots, here these are all down-regulated and compare with control with treated and



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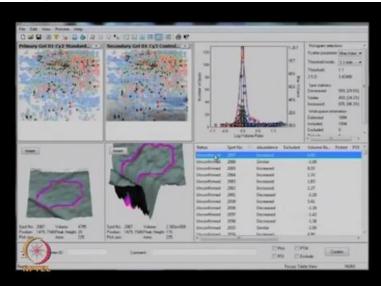
Expert: these blue color spots, there you can see, they are all operated spots when comparing with control and treated. In between these,

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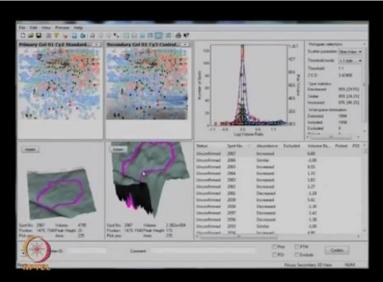
Expert: there is a blue there are some blue color spots. These are all similarly regulated So this is what we can see in DIA. Now you can go through each and individual spot

(Refer Slide Time 03:38)



Expert: and you can see the 3D view of that particular spot

(Refer Slide Time 03:42)



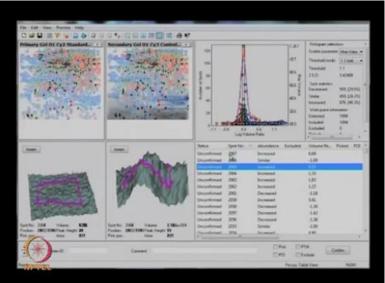
Expert: If suppose, if we can select any...

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Expert: you can go to one by

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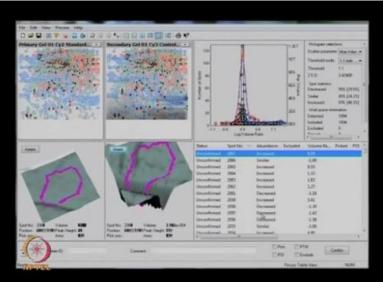
Expert: one and you can see whether it is exactly spot

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Expert: or some background, or if it is a background, you have to remove that So suppose this is the background,

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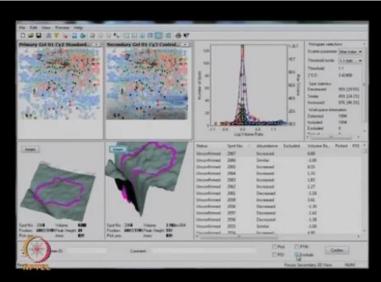
Expert: so there is no spot at all. Still it has detected some background

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| Primary Gel B1 Cy2 Mandard   | Secondary Gel Dx Cy3 Control   | 1 1 1 1 1             |           |                     | 78.87       | Rutagian (etc.)   | citation (     |       |
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Expert: So you can exclude it

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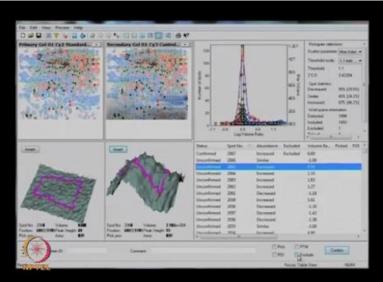
Expert: from here by clicking,

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Expert: then confirm it.

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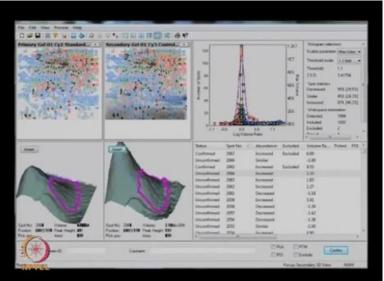
Expert: So this protein has been removed from the gels

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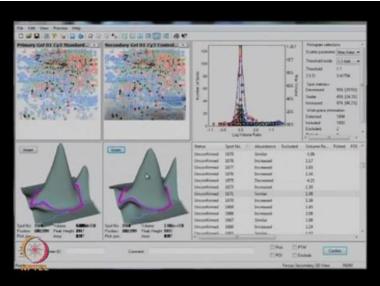
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Expert: So the same way, we can go each and individual,

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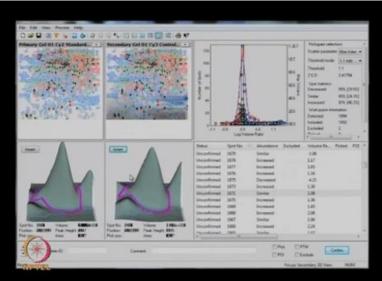
Expert: then exclude it, confirm it. By this way we can check all spots and you can have more accurate data with you like you can see



(Refer Slide Time 04:33)

Expert: how accuracy would be there, like the spot detection

(Refer Slide Time 04:37)



Expert: Now you can see. This is what we will get in DIA.

(Refer Slide Time 04:43)



Expert: This DIA creation we finished

(Refer Slide Time 04:47)



Expert: This is the BVA. BVA is nothing but Biological Variation Analysis One of the Internal Standard image is selected as a master image and all Internal Standard images match into this sample standard spot ratio for each protein. Each sample, then compared giving T-test value, fold changes, ANOVA values for each and individual protein

(Refer Slide Time 05:16)

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Expert: How to create BVA workspace? You can open the BVA and create BVA workspace

(Refer Slide Time 05:23)

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Expert: and go to our DIA workspace where we have our DIAs. From there

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Expert: you can create

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| Multiple particular of applications an appendix to pressure (178), a choice, or pressure (201) a  |

Expert: actually...add it, one DIA I have added and this is another DIA

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|  | Experimental Design View   | Sing             |
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Expert: I am adding here. Minimum 2 DIAs we require for the BVA, so we have 2 DIAs here, so click on create. So it creates a new BVA for you.

(Refer Slide Time 05:55)

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Expert: This is the new BVA. From here, first

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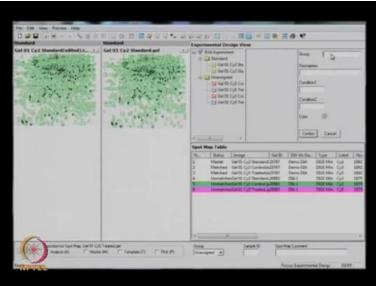
Expert: all Cy2 gels automatically go to Standard folder. There is a Standard folder you can see and

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| Constant and the set of the        | Territori<br>(M) C Territor(C) (C Pos.(P) | In the constant of the constan   |                                |

Expert: remaining all gels remain in the unassigned folder where we need to assign these gels as according to the gel type or sample type. Then you have just have to click on Add option,

(Refer Slide Time 06:28)



Expert: now create a group, it may be control or treated. If first one is the control, and

(Refer Slide Time 06:37)

| Gel HI Cycl Standard edited in 1 | Gel 01 Cyr Mandard gel   | Departmental Decign Vice | Bring Zoood<br>Drampins<br>Carolines  |
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| ACCESSION OF                     | 2.43   |                          | Cantinut<br>Case By<br>Case Cancel  |
|                                  | particular and a second s |                          | Denvilla DEI Min Cyl IBH<br>Servella DEI Min Cyl IBH<br>Denvilla DEI Min Cyl IBH<br>Denvilla DEI Min Cyl IBH<br>DA1 DEI Min Cyl IBH |

Expert: apply some color draft, confirm it,

(Refer Slide Time 06:45)

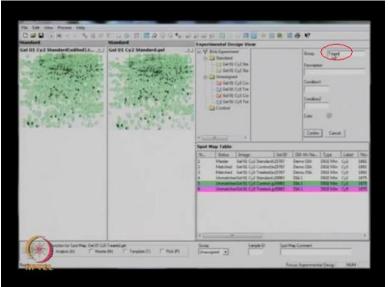
| standard                      | Standard  | Anna pr 12 III III III III III III III III III   |                               |
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| Gel 01 (c/) Shandard edited 3 | V The Appendix of the Appendix Opt The Ap | Concession   |                               |
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|                               |   |  |                               |
| -                             |   | *  |                               |

Expert: then another you can create

(Refer Slide Time 06:47)

|   | Mandard                | an a  |   |
|---|------------------------|---|---|
| Gel 01 Cr Standardteilenind 3 Gel 01 Cr   | Gel 01 (c) Wandows pel | Constant Constant     Constant     Constant   | Breegen   |
|   |                        | Next Map Table  |   |
|   |                        | Matter Gelli Cycl Sanders(2518) DV     Marcheol Gelli Cycl Controlloc25187 DV     Marcheol Gelli Cycl Controlloc25187 DV     Matcheol Gelli Cycl Controlloc25187 DV     Matcheol Gelli Cycl Controlloc25187 DV     Matcheol Cycl Controlloc25187 DV | Dia Han.         Typer         Lation         Hap           BIX Mithod         Cycl         BB4           BIX BIX Mithod         Cycl         BB5           BIX BIX Mithod         Cy |
| Constanting for the first first |                        | in  |   |

(Refer Slide Time 06:51)



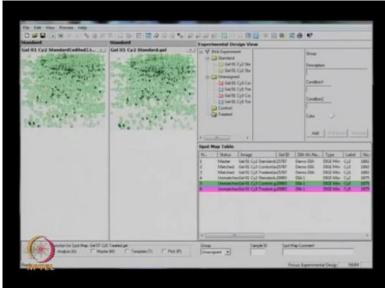
Expert: like treated

(Refer Slide Time 06:56)

| bachard   | Mandard | Coperimental Design V |  |   |
|---|---------|-----------------------|--|---|
| 100 - |         |                       |  | Bree Preser<br>Prompter<br>Cardinal<br>Cardinal<br>Care Carcel<br>Care Carcel   |
|   |         | FFFFFF                | Cyll Sandwat (1578) - Sw<br>Cyll Controlle (1578) - Sw | ma         DM         DML Mos         CpL         DML           ma         DML         DML Mos         CpL         DML         DML           ma         DML         DML Mos         CpL         DML         DML |

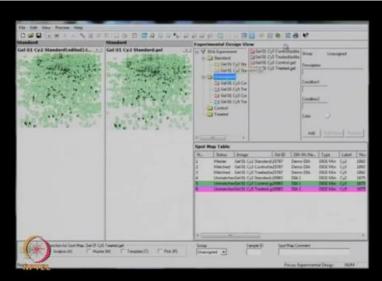
Expert: then give some color draft, confirm it.

# (Refer Slide Time 07:03)



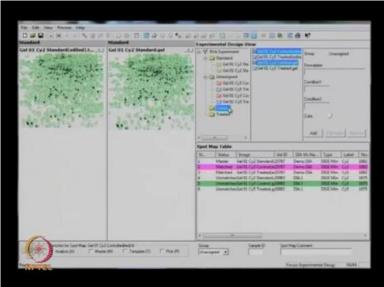
Expert: Now we have 2 folders, confirm and treated. So, as we have in assigned folder

(Refer Slide Time 07:09)



Expert: both control and treated; these control gels we can transfer into Control folder

(Refer Slide Time 07:17)



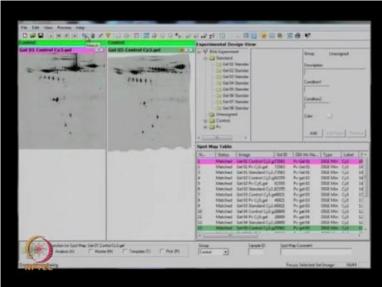
Expert: by dragging those images and treated gels we can transfer to Treated folder by dragging them.

(Refer Slide Time 07:23)

|                                 | 100 T # 2 0 9 4 4   |       |  |   |   |   |  |
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|                                 |   |       | ny Eable<br>Natur   Image  | of Renderf, 2018<br>of Control (2018)<br>in Control (2018)<br>of Renderf (2018) | Data was have<br>Denne Data<br>Denne Data<br>Denne Data<br>Denne Tata<br>Data 3<br>Data 3<br>Data 3<br>Data 3<br>Data 3 | Type Lune<br>Did Me Cyl<br>Did Me Cyl<br>Did Me Cyl<br>Did Me Cyl<br>Did Me Cyl<br>Did Me Cyl | 2004<br>2004<br>2005<br>2005<br>2005<br>2005 |
| Contraction for the sector call | Handadad X<br>M (*** Tragén (*) (*** Pist P)  | e     |  |   | MacConnert  |   |  |

Expert: Now we have our images here.

(Refer Slide Time 07:34)



Expert: After shifting control to Control and treated to Treated, we have to match all gels.

(Refer Slide Time 07:40)

| Justice 1 | and a second  |  | Experimental D                                    | icsign View  |  |   |               |
|-----------|---------------|--|---|--|--|---|---------------|
|           | ZATEL CAMPAGE | -  | An V Bia Igener<br>Standar<br>Gali                | d<br>E Standar<br>E Standar  | Design<br>Design<br>Condition                    |   |               |
|           | •             | March.                                   |   |  | Cardle<br>Caller                                 | <u>=2</u>   |               |
|           | •             | if teachers<br>Constitute<br>Constitutes | ni ani'i ashaini                                  | -  |  |   |               |
|           |               | 97 Openin Harris                         |   | Drage [ Sec<br>and Connect Call of Net<br>and Call of Call of Net | Ar Gerta<br>Ar Gerta<br>Pro Gerta<br>Ar gerta    | - Type Late<br>DKI Min Cyl<br>DKI Min Cyl<br>DKI Min Cyl<br>DKI Min Cyl | T MARK        |
|           |               |  | 1 Matchar<br>8 Matchar<br>9 Matchar<br>20 Matchar | Can 12 Standard Call ACC<br>Electro Carmor Call ACC<br>Electro Carmor Call getter<br>Electro Pr-Call getter<br>Electro Standard Call (Call<br>Carl M Commit Call getter                                  | Av gal 11<br>Av gal 11<br>Av gal 10<br>Av gal 10 | BEEMa Cui<br>BEEMa Cui<br>BEEMa Cui<br>BEEMa Cui<br>BEEMa Cui           | N II II II II |
| 0         |               |  | 12 Matchan  | Gel B Pr Cylingel (2000)<br>Gel B Standard Cyling (2000)<br>Gel B Conner Cyling (2000)   | Augusta .  | DELEMINE Call<br>DELEMINE Call<br>DELEMINE Call                         | Con La Cal    |

Expert: Just click on match and match all. It's matched. The matching process has been finished. Now as we discussed in out of all standard gels, one gel selected as master image

| Gel 01 Control Cy3 pel |   |            | inscental De   |  | -   |         |   |                 |       |      |
|------------------------|---|------------|--|--|---|---------|---|-----------------|-------|------|
|                        | - | 111        | A Standard<br>A Standard<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Control<br>Contro | 1 Jandas<br>1 Jandas<br>1 Jandas<br>1 Jandas<br>5 Jandas<br>5 Jandas<br>1 Jandas<br>1 Jandas | Land II. Bank<br>Seriel Transit<br>Seriel March<br>Seriel March<br>Seriel March<br>Seriel March<br>Seriel March<br>Seriel March |         | Denegal<br>Carolito<br>Carolito<br>Catolito<br>Catolito | a               |       |      |
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|                        |   | 10         |  |  | Collaget 309<br>Involved Cu2,709  |         |   | TRUE Man        |       | 1    |
|                        |   | 14         |  |  | Annual Cyclineers   |         | 1.15  | TRUE MAN        |       | 11   |
|                        |   | 17         |  |  | VOAN EN   |         | 100   | ENCE MAN        |       | 11   |
|                        |   | 10         | Matched  | 64965  | Kansterii Cu7, ATS<br>Control: Cu7, g417  | 8 - Beg | 41 TA   | DOLMA<br>DOLMAN |       | 11   |
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|                        |   | 4          |  |  | taining Cyl. 855  |         | 101   | INCL MAN        |       | - 14 |
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|                        |   | 10         |  |  | 5 Cult-gel - 378  |         | -   | DEL Ma          |       | -11  |
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(Refer Slide Time 07:56)

Expert: as you can see the number 24 gel has been selected as the master and it will compare remaining other gels with this master gel. So now we have this comparison data. After that we need to calculate statistical parameters.

(Refer Slide Time 08:18)

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Expert: So click on Statistical Parameter button. See now we have some statistical parameters like

(Refer Slide Time 08:24)

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Expert: independent T-test

# (Refer Slide Time 08:26)

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|   | General industry and an  | LADON.                                 | Peprill                          | THUE MAY CAT                              | 0    |
|   |  | 41257                                  | Pr pai 17<br>Pr pai 17           | DEEE May Cold                             | 14   |
|   |  | 1.41557                                | Av pail 2                        | SELMe Col                                 | - 14 |
|   |  | 8474/6                                 | Provide                          | DEEMa Cpl                                 | - 14 |
|   |  | 37476                                  | Ft-pail25                        | DELEMAN Cold                              | - 24 |
|   |  | South R.                               | Print and                        | Contra Market State                       |      |
| 12  |  |  |                                  |   | 1    |
| autorite last Mar. Sei III Tanda  | 10 Date: 1 Dat | Spath                                  | Ag Contenti                      |   |      |
| Andrea Million The Andrea Million The   | Calato Calato Mag  |  |                                  |   |      |
| A. 1  |  | 1000                                   | room Tarent Million              |   |      |

Expert: average ratio,

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|                                  | Franker Database<br>Fase & Addated two  | 10 20  | v  |
|----------------------------------|---|--|--|
| Ger D1 Contract (V) ger          | Topolaria Internation     Topolaria     Topolaria | Tendent (G) of<br>Anders (G) of  | December 1   |
|                                  | Melala pina conjuntos<br>IP Daving MON benanc direct prop:<br>↑ Security MON benanc Sedient ant Section<br>↑ Applicate Security of POI constant   | 1080 Fr  | A MIL Nue, Type Laber 17-<br>with DOL Me Got Li<br>with DOL Me Got Li  |
|                                  | falset editor vo a deb  | 42118 Pro<br>42117 Pro<br>42117 Pro<br>42117 Pro<br>42117 Pro<br>42117 Pro<br>42117 Pro<br>42118 Pro | and DEFMA CALL<br>and DEFMA CALL |
| Contractor Lar Mar Lar II Towned | Latan (and the  | Parma C  | anati<br>Iani Mas Tatio - NEMI   |

Expert: Student T-test

(Refer Slide Time 08:30)

| Fir And Date Process long  | Polen Debuto   |             |                    | _                        |      |
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| Castrof 236  | * Independent levis (normal)   |             |                    |                          |      |
| Gel 01 Control Cylippi 8_1 Gel   | C Pandam (see careb E)   | - Bandard C | April (Sample      | Trainbart.               |      |
| and the second division of the second divisio | ( Contraction of the Contraction | Sundard C   | A set              | Concept.                 |      |
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| and the second se  |  |             | Case               |                          |      |
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|  |  |             |                    | Sec. Bar                 |      |
|  | Distant Distant  |             | - 48               | C. Silving 3             |      |
|  |  | -           |                    |                          |      |
|  | Ridge pro company  |             |                    |                          |      |
| and the second se  | C David a Millin Inner Band parts  | 1640        | CDA HIL NA         |                          |      |
|  |  | 1,000       | Avge18             | DELMA CA                 | - 14 |
|  | 7 Tuesday All Tel Innues Medical and Condinal  | 104         | Property 1         | THE MAN CAN              | -    |
|  |  | 1,71942     | Nott               | TRUE Man Call            | 11   |
|  | T Apph false discourse (also PDR) considers  | ad total    | Pupping            | DELLARS CAR              | 1.1  |
|  | Second manufact and the  | (max        | Fr pri DE          | DEEMs. Cyl.              | 17   |
|  | Concert Dates of the Longer  | 4.47156     | Num                | THUR MAY, SUIT           | 0    |
|  |  | 41357       | Pepelt             | DOEM# C15                | - 15 |
|  |  | 400         | Pripill<br>Pripill | Did Ma Cul<br>Did Ma Cul | 비    |
|  |  | 417476      | Print              | DELE MAN Call            | - 12 |
|  |  | 17476       | Pagel 28           | CHild Man Cull           | - 52 |
|  |  | COMPANY.    | Po gal 10          | State Man Call           | 14   |
|  |  |             |                    |                          |      |
| 1  | 4 / ·  |             |                    |                          | -    |
| Con- Andrea Star Ser 18 Transler   | Cause Cause Hay  | 200         | MacConnent         |                          |      |
|  |  |             |                    |                          |      |

Expert: one way ANOVA in between different groups We are doing between control and treated. So calculate them.

(Refer Slide Time 08:39)

| Fat Lat time Annual 1989   | Potent Deliving 270 (ML.200  |   |   |
|--|--|---|---|
|  | - Tan it easterstem  | 10 20   | N7  |
| Call OI Control (V) and<br>24101 Control (V) an | Progets good competence<br>Progets good competence<br>Progets from<br>Progets 1<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets<br>Progets | Annales (G) pri<br>Andres (G) pri<br>Annales (G) pri<br>Annales (G) pri<br>Annales (G) pri<br>Annales (G) pri | December  |
|  | Princips Ethiose     Princips     Princ  | 1,2000 Pro<br>1042 Pro<br>1042 Pro<br>1,2042 Pro<br>1,2042 Pro<br>1,2042 Pro                                  | a Militan Type Later I /<br>prist 200 Mile Col Li<br>prist 200 Mile Col Li  |
|  | Select rain d'un a des   | 42118 Pro<br>94210 Rep<br>42207 Rep<br>42207 Rep<br>42207 Rep<br>42208 Rep                                    | prink Dict Mar Caf ET<br>prink Dict Mar Caf ET<br>prink Dict Mar Caf ET<br>print Dict Mar Caf Et |
| Carporter to Los Mai Los III Tandel  |  | -   | annant<br>Iant Maa Takka : MURA   |

Expert: So statistical parameters has been calculated

(Refer Slide Time 08:45)



Expert: Now we can see exact results of statistical parameters. If I can go to the table view

(Refer Slide Time 08:56)

| Protein Table           | T-bist and | d Au Rathi | Py / Control                                   |          |          |          |                |                |                            |    |
|-------------------------|------------|------------|--|----------|----------|----------|----------------|----------------|----------------------------|----|
| Pan Put                 | Puistle    | Manar_     | I thena Approximite                            | Cas      | Say fate | 11-4MD-  | 2-MENA-Could . | T 2 4MCNA Cant | R. [ 2 ANDUA Descent ] PCS |    |
| \$102 ·····             |            | 37         | Decelonatil (H) T                              | 2017 14  |          | 6403     |                |                | 1                          |    |
| 200                     |            | 35         | Decodormal B (24) 1                            | 8/202    | 1.40     | 682      |                |                |                            | 14 |
| 82                      |            | 31         | UncardiomadEl (26) 1                           | 8245     | 1.14     | 0.045    |                |                |                            |    |
| (A)()                   |            | 34         | Disjoherad22-(24) 1                            | 8452     | 1.42     | 0.012    |                |                | 1                          |    |
| 3.1                     |            | 40         | Departments (30) 1                             | 0.00054  | 1.07     | 0,0004   |                |                | . I                        |    |
| (K)                     |            | - 11       | Development (20) Y                             | 1.00070  | LB       | 0.000276 |                |                | 1                          |    |
| K21                     |            |            | Uncolored DD 7                                 | 1.0148   | 1.45     | 0.1049   |                |                | 1                          |    |
| NO.                     |            | 48         | SecondineedS (54) 1                            | 1.048    | 1.87     | 5.048    |                |                |                            |    |
| (A)                     |            | .0         | Department24 (24) T                            | 1.010    | 2.51     | 0.0038   |                |                | 21                         |    |
| 10                      |            | 72         | University of the T                            | 4,0179   | 254      | 0.0079   |                |                |                            |    |
| 0.2                     |            | 17         | Decement21 (24) T                              | 0.0040   | 1.72     | 0.7040   |                |                |                            |    |
| 121                     |            |            | Unconformation (24) 1                          | 324-805  | 1.84     | 5.24.005 |                |                | 1.1                        |    |
| 41                      |            | 378        | Shuteferrad24 (24) 1                           | 1032     | 1.28     | 8.856    |                |                | 1.1                        |    |
| 34                      |            | 111        | December and (D) 1                             | 8.84-208 | 285      | 2.4+-005 |                |                |                            |    |
| 33                      |            | 1/1        | Deuxforma(24.06.1                              | 0.0038   | 3.45     | 0.0039   |                |                |                            |    |
| H                       |            | 3.00       | Linconformatile (14). 1                        | 0.044    | 4.8      | 0.044    |                |                |                            |    |
| 17                      |            | 10.        | Geosterent2 (34) ?                             | 0.0029   | 3.42     | 0.0025   |                |                |                            |    |
| 14                      |            | 138        | December (A) 7                                 | 8.012    | 4.38     | 8.812    |                |                |                            |    |
|                         |            |            | Unconfictual/M (34) T                          | 8.013    | 1.33     | 8.813    |                |                |                            |    |
| 8                       |            | 154        | Electrofferme(24 (24), T                       | 1.005    | 1.37     | 0.0029   |                |                |                            |    |
| a                       |            | 135        | Deperments (30, 7                              | 8,0079   | 3.37     | 0.9670   |                |                |                            |    |
| 8                       |            | 345        | Desperimental (24) T                           | 8,039    | 4.51     | 108      |                |                |                            |    |
| 32                      |            | 384        | Departmental (20 1                             | 824-200  | 3.00     | 3.34-308 |                |                |                            |    |
| 24                      |            | 100        | Uncoderadit (30) 7                             | 8.00     | Lat      | 0.025    |                |                |                            |    |
| 25                      |            | 287        | Second and Sold T                              | 8,00002  | 2.22     | 0.000030 |                |                |                            |    |
| <u>a</u>                |            | 385        | Unconferencial (36) 1<br>Unconferencial (36) 1 | 0.001    | 1.49     | 0.0022   |                |                |                            |    |
| 2                       |            | 184        | ShedreformadDie (34) T                         | 0.011    | 1.36     | 0.013    |                |                |                            |    |
| 8                       |            | 100        | Deconfirmed/4 (24) 1                           | 8,015    | 1.48     | MIN      |                |                |                            |    |
| 2                       |            | 347        | Unconformed(3 (20 7                            | 0.0045   | 1.40     | 0.0045   |                |                |                            |    |
| 8                       |            | 105        | Decentered UK 7                                | 0.03     | 1.00     | 0.0045   |                |                |                            |    |
| 20                      |            |            |  |          |          |          |                |                |                            |    |
| * Suntann               |            |            |  |          |          |          |                |                |                            | 1  |
| No. of Concession, Name |            | 252        | Unconformed R DK 1                             | 1027     | 1.59     | 1427     |                |                |                            |    |

Expert: here you can see T-test

(Refer Slide Time 09:00)

| Dial Processor         Dial Pr   | a temp   |   |           |
|--|--|---|-----------|
| Pro:         Protection         Mater.         Res.         Appropriet         1         Support         T         Support         Support </th <th></th> <th>2 12 13 13 13 13 13 13 13 13 13 14 14 14 14 14 14 14 14 14 14 14 14 14</th> <th></th>  |  | 2 12 13 13 13 13 13 13 13 13 13 14 14 14 14 14 14 14 14 14 14 14 14 14  |           |
| 1         11         Barellmentill (k)         7         40.0         1.0         <  | and the second |   |           |
| 1         0  |  |   | AND POL > |
| 1         Bits of March 1000 00 0 0 0000 0000 0000         10000000000         100000000000         100000000000         1000000000000         1000000000000000000000000000000000000   |  |   | 1.2       |
| 4         M         Beardment 50 (0)         1         0.001         1.42         USD           6         7         6         6         7         6 <td></td> <td></td> <td></td>  |  |   |           |
| 1         0         Descrimental (0, 1)         10001         101         10001         10001         101011         101011  |  |   | 1.1       |
| B         Hi         Description(2) (2)         T         BB(R)         L.8         DB(R)           H         Description(2) (2)         1         BB(R)         1.6         DB(R)           H         Description(2) (2)         1         BB(R)         1.6         DB(R)           H         Description(2) (2)         1         BB(R)         1.64         DB(R)           H         Description(2) (2)         1         BB(R)         1.64         DB(R)           H         Description(2) (2)         1         BB(R)         2.64         DB(R)           H         Description(2) (2)         1         3.24         DB(R)         2.7         DB(R)           H         Description(2) (2)         1         3.24         DB(R)         2.8         DB(R)           H         Description(2) (2)         1         3.24         DB(R)         2.8         DB(R)           H         Description(2) (2)         1         3.24         DB(R)         1.8         DB(R)           H         Description(2) (2)         1         DB(R)         1.8         DB(R)           H         Description(2) (2)         1         DB(R)         DB(R)         DB(R)  |  |   |           |
| 1         Hit         Description(2) (1)         7         6580         1.67         5080           0         40         Description(2) (2)         7         6180         1.67         5080           0         40         Description(2) (2)         7         6180         1.67         5080           0         40         Description(2) (2)         7         6180         2.61         5080           10         Description(2) (2)         7         6180         1.41         5080           11         Description(2) (2)         7         6180         1.23         5180           11         Description(2) (2)         7         6180         1.41         5020           12         Description(2) (2)         7         6180         1.41         5020           13         Description(2) (2)         7         6181         1.41         5020           13         Description(2) (2)         7         6181         1.41         5020           14         Description(2) (2)         7         6181         1.41         5020           15         Description(2) (2)         7         6181         1.42         5080           15         Descri   |  |   | 1.1       |
| B         B         Beachmeet 24 7         0.00         1.07         0.000           0         Beachmeet 24 7         0.000         1.00         0.000           10         Beachmeet 24 7         0.000         2.00         0.000           11         Beachmeet 24 7         0.000         2.00         0.000           11         Beachmeet 20 7         0.000         2.00         0.000           11         Beachmeet 20 7         0.000         2.00         0.000           12         Beachmeet 20 7         0.000         1.00         0.000           13         Beachmeet 20 7         0.000         1.00         0.000           14         Beachmeet 20 7         0.000         1.00         0.000           15         Beachmeet 20 7         0.000         1.00         0.000           16         Beachmeet 20 7         0.010         1.00         0.000           16         Beachmeet 20 7         0.012         1.00         0.000           17         Beachmeet 20 7         0.012         1.00         0.000           16         Beachmeet 20 7         0.012         1.00         0.000           17         Beachmeet 20 7         0.01  |  |   |           |
| B         B         Generalmental (10)         T         4000         154         0.001           11         12         Generalmental (10)         T         0.001         22         0.000           13         17         Generalmental (10)         T         0.000         22         0.000           14         0.001         1.0.000         2.0         0.000         0.000         0.000           15         10         0.000         1.0.000         2.0         0.000         0.000           16         0.000         1.0.000         2.0         0.000         0.000         0.000           16         0.000         0.000         1.0.000         0.000         0.000         0.000           16         0.000         0.000         1.0.000         0.000         0.000         0.000           17         0.000         0.000         1.000         0.000         0.000         0.000           18         0.000         0.000         1.000         0.000         0.000         0.000           19         0.000         0.000         1.000         0.000         0.000         0.000           10         0.000         0.000         1.   |  |   |           |
| 10         17         Discretificação (2)         7         Alter (2)         5000           12         10         10         10         10         10         10           13         10         10         10         10         10         10         10           13         10         10         10         10         10         10         10           14         10         10         10         10         10         10         10           14         10         10         10         10         10         10         10           15         10         10         10         10         10         10         10           16         10         10         10         10         10         10         10           17         10         10         10         10         10         10         10           18         10         10         10         10         10         10         10           19         10         10         10         10         10         10         10           19         10         10         10         10   |  |   |           |
| 13         17         Biosoffmed2 (2) T         3.24 (8)         2.22         5.040           14         14         Biosoffmed2 (2) T         1.24 (8)         5.040         5.040           15         14         3.040         1.24 (8)         5.040         5.040           15         14         3.040         1.24 (8)         5.040         5.040           16         14         5.000         1.24 (8)         5.000         5.000           16         15         Biosoffmed2 (2) T         1.040 (8)         5.000         5.000           16         Biosoffmed2 (2) T         1.040 (8)         1.04 (8)         5.000         5.000           17         Biosoffmed2 (2) T         1.040 (8)         1.02 (8)         5.000         5.000           18         Biosoffmed2 (2) T         1.020 (8)         1.01 (8)         5.000         5.000           18         Biosoffmed2 (2) T         1.020 (8)         1.01 (8)         5.000         5.000           19         Biosoffmed2 (2) T         1.020 (8)         1.000 (8)         5.000         5.000           10         Biosoffmed2 (2) T         1.021 (8)         1.000 (8)         5.000 (8)         5.000 (8)           10   |  |   |           |
| Bit         Bits outward (10)         C         1.34         1.54         1.34         3.34         1.34         3.34         1.34         3.34         1.34         3.34         1.34         3.34         1.34         3.34   | 12 Chargentine EN (24) 7 8,007   |   |           |
| 11         11<   |  |   |           |
| III.         Uncertinged UP         III.         III.         Uncertinged UP         III.         III.         IIII.         Uncertinged UP         III.         IIII.         IIII.         IIII.         IIII.         IIII.         IIII.         IIIII.         IIIII.         IIIII.         IIIII.         IIIII.         II   |  |   |           |
| III         Lize Insurfaced 201 7         VID/0         VI   |  |   |           |
| Mit         1.3         Househmedia (2)         1         Hat         1.4         Dist         1.4         Dist           11         13         Househmedia (2)         1         0.01         1.4         Dist         0.01         1.4         Dist         0.01         1.4         Dist         Dist         0.01         1.4         Dist         Dist <td< td=""><td></td><td></td><td></td></td<>   |  |   |           |
| 11         110         HouseffeedB2 (1)         1         4.00         1.42         0.003           11         110         HouseffeedB2 (1)         1         0.01         1.01         0.01           12         HouseffeedB2 (1)         1         0.01         1.11         0.00         0.01           13         HouseffeedB2 (1)         1         0.01         1.11         0.00         0.01           13         HouseffeedB2 (1)         1         0.010         1.11         0.000         0.010  |  |   |           |
| Image: State State         Topology         Topology <thtopology< th=""> <thtopology< th=""> <thtopology< th=""></thtopology<></thtopology<></thtopology<>   |  |   |           |
| B         L12         Investmential (2): 7         111         L1         Mull           154         Uncestmential (2): 7         8405         5.57         5025           21         Uncestmential (2): 7         8405         5.57         5025           22         124         Uncestmential (2): 7         1.88         5.88           24         Uncestmential (2): 7         1.88         5.88           25         Uncestmential (2): 7         1.88         5.88           26         Uncestmential (2): 7         4.88         5.88           27         1.88         Uncestmential (2): 7         4.88         5.88           28         Uncestmential (2): 7         4.88         5.88         5.88           28         Uncestmential (2): 7         5.81         5.88         5.88           29         186         Uncestmential (2): 7         5.81         5.88         5.88           29         186         Uncestmential (2): 7         5.81         5.88         5.82           20         Uncestmential (2): 7         5.81         5.88         5.82           20         Uncestmential (2): 7         5.81         5.87         5.88           21         Uncestme   |  |   |           |
| B         154         UseraffeedB 10 f         F         800 f         137         000 f           B         155         UseraffeedB 10 f         830 f         137         000 f           B         144         UseraffeedB 10 f         848 f         4.33         6.68           B         144         UseraffeedB 10 f         848 f         148         5.68           B         144         UseraffeedB 10 f         6.68         1.68         5.696           B         146         UseraffeedB 10 f         6.68         1.68         5.696           B         UseraffeedB 10 f         0.601 f         1.68         5.696           B         UseraffeedB 10 f         1.691 f         1.68         5.696           B         UseraffeedB 10 f         1.691 f         1.68         5.696           B         UseraffeedB 10 f <t< td=""><td></td><td></td><td></td></t<>   |  |   |           |
| State         Line of leveral model (2):         T         DB/ID         Line (2):         DB/ID         DB/ID <thdb id<="" th="">         DB/ID         DB/ID         &lt;</thdb>   |  |   |           |
| 21         24         Osconference[2] (2)         1         28         4.3         8.6           28         144         Unconference[2] (2)         1         8.6         8.6         8.6           28         144         Unconference[2] (2)         1         8.6         8.6         8.6           28         145         Unconference[2] (2)         1         8.6         8.6         8.6           28         146         Unconference[2] (2)         1         8.6         1.6         8.6           28         146         Unconference[2] (2)         1         8.6         1.6<   |  |   |           |
| Bit         Bit         Descriptions         Display         Display <thdisplay< th=""> <thdisplay< th=""> <thdisp< td=""><td></td><td></td><td></td></thdisp<></thdisplay<></thdisplay<>  |  |   |           |
| 36         146         Hexadinesi21 (2)         7         610         1.80         0.00           36         140         Hexadinesi21 (2)         7         610         1.80         0.00           37         140         Hexadinesi21 (2)         7         610         1.80         0.00           38         140         Hexadinesi21 (2)         7         610         1.80         0.00           39         140         Hexadinesi21 (2)         7         610         1.80         0.00           317         Hexadinesi22 (2)         1         610         1.60         0.00         0.00           318         Hexadinesi22 (2)         1         1.90         1.60         0.00         0.00           317         Hexadinesi22 (2)         1         1.90         1.60         0.00         0.00           318         Hexadinesi22 (2)         1         1.90         1.60         0.00         0.00           318         Hexadinesi2 (2)         1         1.90         1.80         0.00         0.00           318         Hexadinesi2 (2)         1         1.90         1.80         0.00         0.00         0.00           318         Hexadin  |  |   |           |
| Bit         Unconferential (2)         1         4 (2010)         2.22         100000           Bit         State International (2)         7         1.64         1.69         1.60           Bit         Deconferential (2)         7         1.61         1.54         5.60           Bit         Deconferential (2)         7         1.54         5.60         5.60           Bit         Deconferential (2)         7         1.54         5.55         5.60           Bit         Deconferential (2)         7         1.61         1.54         5.60           Bit         Deconferential (2)         7         1.64         5.60         5.60           Bit         Deconferential (2)         7         1.64         5.60         5.60           Bit         Deconferential (2)         7         1.64         5.60         5.60           Bit         Deconferential (2)         7         5.61         1.68         5.60           Bit         Deconferential (2)         1.62         1.68         5.60         5.60           Bit         Deconferential (2)         1.62         1.68         5.60         5.60         5.60  |  |   |           |
| B         140         Use offenessiti (2)         1         140         0.002           27         180         Use offenessiti (2)         1         1.0         0.002           28         184         Use offenessiti (2)         7         0.01         1.4         0.02           28         184         Use offenessiti (2)         7         0.01         1.4         0.02           29         186         Use offenessiti (2)         7         0.01         1.4         0.00           20         186         Use offenessiti (2)         7         0.01         1.4         0.00           20         Use offenessiti (2)         7         0.01         1.4         0.00         0.00           21         Use offenessiti (2)         7         0.01         1.8         0.00         0.00           21         Use offenessiti (2)         7         0.01         1.8         0.02         0.00           21         Use offenessiti (2)         1         0.02         7         0.02         0.00           21         Use offenessiti (2)         1         0.02         7         0.02         0.00   |  |   |           |
| 27         18         Decemberation (2)         1         1.04         1.04         0.01           38         184         Decemberation (2)         1         1.14         0.02         0.01           39         184         Decemberation (2)         1         1.14         0.02         0.01 <td></td> <td></td> <td></td>  |  |   |           |
| St         184         OwnerHenneski (2) (2)         7         101         1.4         0.051           St         186         OwnerHenneski (2)         7         0.01         1.41         0.001           St         190         OwnerHenneski (2)         7         0.01         1.41         0.001           St         190         OwnerHenneski (2)         7         0.001         1.44         0.001           St         190         OwnerHenneski (2)         7         0.001         1.44         0.001           St         190         OwnerHenneski (2)         1         0.001         1.44         0.001           201         Descriptional (2)         1         0.001         1.44         0.001           202         Descriptional (2)         1.402         1.44         0.001         0.001           203         Descriptional (2)         1.402         1.44         0.001         0.001           203         Descriptional (2)         1.402         1.403         0.001         0.001  |  |   | 100       |
| St         100         Deconfirmed(2) (0, 1         6.013         1.41         6.000           St         107         Local Prince (2) (1         1.600         1.600         1.600           St         106         Deconfirmed(2) (0, 1         1.600         1.600         1.600           St         106         Deconfirmed(2) (0, 1         1.601         1.500         1.600           St         1.60         Deconfirmed(2) (0, 1         1.602         1.500         1.602           T         1.60         Deconfirmed(2) (0, 1         1.602         1.500         1.600   |  |   | 100       |
| B         307         Decodered 201.7         1001         146         6800.           10         Decodered 201.7         1001         146         6800.           20         Decodered 201.7         1001         146         146           21         Decodered 201.7         1507         159         1507           22         Decodered 201.7         1507         159         1507   |  |   | 1         |
| B         10         Devolvement2 (0)         1401         136         8431           20         20         20         20         20         20         20           7         20         20         20         20         20         20         20           9         20         20         20         20         20         20         20   |  |   | 1         |
| 22 December 201 1 1427 138 1527  |  |   |           |
| Contar Assis Asses Sever a Trave Cas Martin  |  |   | 1 .       |
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| and the second s |  | PIM CPuk CI Lui   | Durge 54  |
|  | Sence (1979  | a family of the second |           |
| Party Annual   |  |   |           |

Expert: one way ANOVA, this you can see.

## (Refer Slide Time 09:01)

| er. T-tesit an | ed Au Ratio                | e Py / Cust  | best .  |   |  |   |  |   |  |  |  |   |
|----------------|----------------------------|--|---|---|--|---|--|---|--|--|--|---|
| Puted In       | Madae                      | there :  | Appendix  | 144   |  |   | 2 AND A Could.   | 2.44014   | Conda, 12  | AND NA SHOWN   | 104  |   |
|                | 17                         |  |   |   |  |   |  |   |  |  | 1  |   |
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|                |                            |  |   |   |  |   |  |   |  |  | 1  |   |
|                |                            | Charlestone  | 1. (KG-Rither   | 8.00012   | 2,22   | 0.00002   |  |   |  |  | 1  |   |
|                |                            |  |   |   |  |   |  |   |  |  |  |   |
|                |                            |  |   | 1068  |  |   |  |   |  |  | 1  |   |
|                |                            |  |   | 0.025   | 1.54   |   |  |   |  |  | 1  |   |
|                | 100                        |  |   | -0.015  | 3.45   |   |  |   |  |  | 1  |   |
|                | 3.67                       | Department   |   | 93045   | 5.40   | 0.0045  |  |   |  |  | 1  |   |
|                | 125                        | Desidence  | 1.041833  | 8-051<br>8-027  | 138  | 0.012   |  |   |  |  | 1  |   |
|                | 201                        | Destantions  |   |   |  |   |  |   |  |  |  |   |
|                | the state of the latter is | Printfill         Using           17         17           18         18           19         18           10         17           11         18           12         19           13         18           14         19           15         19           16         19           17         19           18         19           19 | Prior Di         Modes         Mass           1         1         Status           1         Status         Sta | Polastica         Mater.         Mater.         Spectrum           11         Viscontranuelli di Control         1           12         Viscontranuelli di Control         1           13         Viscontranuelli di Control         1           14         Viscontranuelli di Control         1           14         Viscontranuelli di Control         1           14         Discontranuelli di Control         1           14         Discontranuelli di Control         1           15         Discontranuelli di Control         1           16         Discontranuelli di Control         1           17         Discontranuelli di Control         1           18         Discontranuelli di Control         1           19         Discontranuelli di Control         1           10         Discontranuelli di Control         1           11         Discontranuelli di Control         1           11         Discontranuelli di Control         1           11         Discontranuelli di Control         1           12         Discontranuelli di Control         1           13         Discontranuelli di Control         1           14         Discontranuelli di Control         1 </td <td>Point III         Nature         Nature         Spannessee         Test of the spannessee           1         Occonformal 0.01         50.1         60.0           1         Descriptional 0.01         60.1         60.0           1         Descriptional 0.01         60.0         60.0           1         Descriptional 0.01         60.0         60.0           10         Descriptional 0.01         60.0         60.0           10         Descriptional 0.01         10.0004         60.0           10         Descriptional 0.01         10.0004         60.0         60.0           10         Descriptional 0.01         10.0004         60.0         60.0         60.0           10         Descriptional 0.01         10.0004         60.0</td> <td>Polastin         Mates         Spectrum         Test         As lational state of the sta</td> <td>Point District         Instant         <thinstant< th="">         Instant         <thinstant< th=""></thinstant<></thinstant<></td> <td>Polastin         Mater.         Supervisor         Test         A. fore         L. M. 1         J. J. M. 10.         J. M. 10.</td> <td>Polastin         Mass         Spannerse         Test         As Batis         Label:         2 AddDia           11         Decomberned         0.0</td> <td>Polastin         Meson         Experiment         Total         4.1 Mark         2 AddChik Condet.         7 AddChik Condet.         <th7 addchi<="" td=""><td>Polastin         Mater.         Spenner:         Test         As Bate         Label:         2 AddDia Count.         2 AddDia Count.</td><td>Polastin         Mass         Spprome         Test         As Bate         Laberts         2 AddNA Coulds         1 AddNA Coulds         <th1 addna="" coulds<="" th=""> <th1 addna="" coulds<="" th=""></th1></th1></td></th7></td> | Point III         Nature         Nature         Spannessee         Test of the spannessee           1         Occonformal 0.01         50.1         60.0           1         Descriptional 0.01         60.1         60.0           1         Descriptional 0.01         60.0         60.0           1         Descriptional 0.01         60.0         60.0           10         Descriptional 0.01         60.0         60.0           10         Descriptional 0.01         10.0004         60.0           10         Descriptional 0.01         10.0004         60.0         60.0           10         Descriptional 0.01         10.0004         60.0         60.0         60.0           10         Descriptional 0.01         10.0004         60.0 | Polastin         Mates         Spectrum         Test         As lational state of the sta | Point District         Instant         Instant <thinstant< th="">         Instant         <thinstant< th=""></thinstant<></thinstant<> | Polastin         Mater.         Supervisor         Test         A. fore         L. M. 1         J. J. M. 10.         J. M. 10. | Polastin         Mass         Spannerse         Test         As Batis         Label:         2 AddDia           11         Decomberned         0.0 | Polastin         Meson         Experiment         Total         4.1 Mark         2 AddChik Condet.         7 AddChik Condet. <th7 addchi<="" td=""><td>Polastin         Mater.         Spenner:         Test         As Bate         Label:         2 AddDia Count.         2 AddDia Count.</td><td>Polastin         Mass         Spprome         Test         As Bate         Laberts         2 AddNA Coulds         1 AddNA Coulds         <th1 addna="" coulds<="" th=""> <th1 addna="" coulds<="" th=""></th1></th1></td></th7> | Polastin         Mater.         Spenner:         Test         As Bate         Label:         2 AddDia Count.         2 AddDia Count. | Polastin         Mass         Spprome         Test         As Bate         Laberts         2 AddNA Coulds         1 AddNA Coulds <th1 addna="" coulds<="" th=""> <th1 addna="" coulds<="" th=""></th1></th1> |

Expert: So we can select from here which are all the statistically significant and which are not significant.

(Refer Slide Time 09:11)

| Get 01 Cycl Control get  | Get 02 CyS Treated.get   | /   |
|--|--|---|
| · ····································   | · ····································                             | -   |
| T. AN CO   | apress. 9  | 10000   |
| Gel 03 Cy's Control pel  | Get 02 Cy3 Treated get   | Contraction of the second                                     |
|  | (BNT)  | Distance (20) Master for UAC Pro-                             |
| Gel 03 Cy3 Control of  | Gel 03 (y) Treatelant  | - Contraction   |
| Call D4 CyS Control gal  | · Sector   | Dit for 130 Marie file 130 Pie.<br>Graph View - Marker No. 1. |
| C. States  | Charles and  | 1.  |
| Appearance Table Martar No. 1242<br>N. Image Get21 204 No. 234 No. Type                                      | Laber   Function   Log Md Allen.   Sel Allen.   Volume   Paul Pb - |   |
| A Gerth Cold Terrent Mill 170 Gerth Child Child Advert<br>A Gerth Cold Conscional 170 Gerth Child The Advert | Cal 444 4.36 10079 450<br>Cal 813 Lat 5469 1714                    |   |

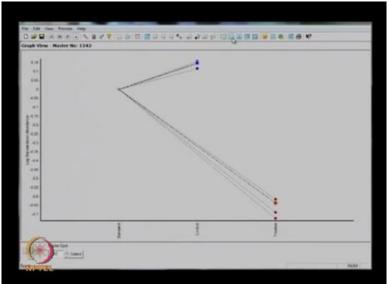
Expert: After analyzing the statistical data, now we can see the complete results here. Here we can see the four views

(Refer Slide Time 09:20)

| Gal B S Cyll Control and | Coll Of Cyn Trendd arl   | •.4         |
|--------------------------|--------------------------|-------------|
| Gel 92 Cys Control per   | GERD CAT Treaded and     | •           |
| Gel 83 Cy3 Control gel   | Gel G3 Cys Treated gel   | <b>ن</b> .• |
| Cel D4 Cy5 Control.pet   | Get Bek Cys Treasted get | د.•         |

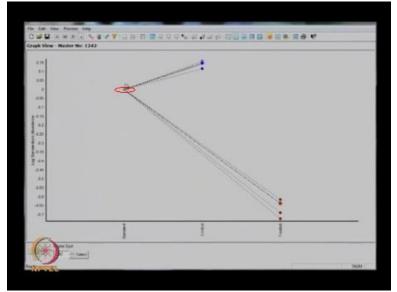
Expert: like this is the image view

(Refer Slide Time 09:22)



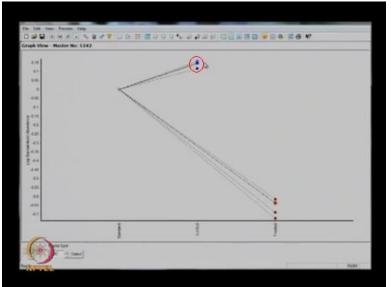
Expert: And this is the histogram view. In histogram view we can see clearly particular protein, how it is behaving throughout control and treated. We can see this is the standard gel, that means this is the mixture of control and treated, this is

(Refer Slide Time 09:39)



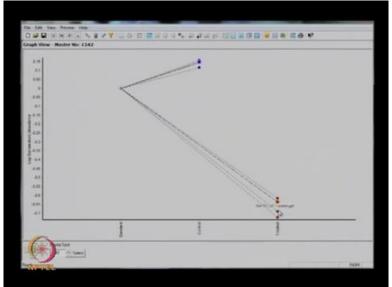
Expert: somewhere zero, we can consider this one. Then control is completely...

(Refer Slide Time 09:39)



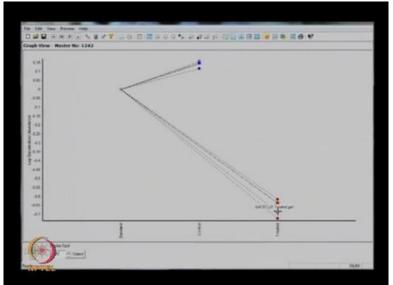
Expert: it is showing up-regulation and

(Refer Slide Time 09:47)



Expert: after giving the particular treatment, it is showing the down-regulation.

(Refer Slide Time 09:50)



Expert: So this kind of data we can see here, then in the table view

(Refer Slide Time 09:57)

| Appea  | invenie Table                        |          | ander No. | 1343        |            |     |           |              |            |          |              |                       |                |
|--------|--------------------------------------|----------|-----------|-------------|------------|-----|-----------|--------------|------------|----------|--------------|-----------------------|----------------|
| N      | linepr.                              | liet D   | 1254-500  | THA WE FIR. | Type       |     | I.Fundant | Log Tel Adu. | The Album. | 2 Datume | Peak Insight |                       | Comp Descentes |
| 1.0    | Gat 10, Cyl Har                      |          | \$387     | -Celli      | DOI Me     |     |           | 3.85         | 3.00       | 201148   | 1878         | Standard              |                |
| 2.     | Bell CylCor                          |          | 1.087     | Gefft.      | DIGEMAN    |     |           | 3.24         | 1,38       | 297122   | 1258         | Control               |                |
| K      | Barritt CyS Tree                     |          | 1287      | Sell.       | TOGR MAN   |     |           | 4.72         | -529       | 110917   | 184          | Treatest              |                |
| 4      | Sec 102 Cold Star                    |          | 101       | 9492        | DIGE More  |     |           | LH           | 2.000      | 311046   | 1730         | Handard               |                |
| 1.1    | Events Cyst Tree                     | PAG 2011 | 100       | GARD .      | DOL MAN    |     |           | -1.84        | -4.34      | 110/56   | 458          | Total .               |                |
| 1.000  | Ser 10 Call Cas                      |          | 101       | Cellin.     | DRIE MAN   | 1.0 | Co        | 8.02         |            | -        | 1794         | Castrol .             |                |
| 100    | 10+101.052 Net                       |          | 8753      |             | THUE MAN   |     | -         | 2.00         | 1.00       | 2012134  | 383          |                       |                |
| 2      | Sel 63 Cyll Carl<br>Gel 63 Cyll Tree |          | 1001      | CHED .      | DBUE Mile. |     |           | 10           | 4.00       | 142945   | 100          | Control .<br>Treatest |                |
| 12 - I | Gerild Cult Star                     |          | 1274      | 649         | DOLL MAN   |     |           | 1.00         | 1.00       | 30372    | 100          | Mandard .             |                |
| 8      | Get Dir Cult Tree                    |          | 1224      | Galla       | CHIE MAN   |     |           | 440          | 414        | 111473   | ME           | Tested                |                |
| ÷.     | Salibe Cut Can                       |          | 1214      | Lafe .      | DIGE NEW   |     |           | 0.28         | 1.84       | CARDINE. | 1211         | Contract              |                |
|        |                                      |          |           |             |            |     |           |              |            |          |              |                       |                |
| 19     | - New Spec                           |          |           |             |            |     |           |              |            |          | _            |                       |                |

Expert: as we can see the complete protein data

#### (Refer Slide Time 10:03)

| 1.00     | vin Table | T-tenz an | d Av Roth | : Treated / | Control  | -           |          |            |                 |              |                      |     |   |
|----------|-----------|-----------|-----------|-------------|----------|-------------|----------|------------|-----------------|--------------|----------------------|-----|---|
| Pas.     | Pus       | Pulsed In | Matter    | Inna        | Appendix | Cine >      | Au. Same | 1 AND      | 7 AMONA Conditi | T ANOTA Cred | 1. 1.2 40214 Descent | 104 |   |
| 41       |           |           | 348       | Cardimad    |          | 1 March     | 3.04     | 0.00018    |                 |              |                      | 1   |   |
| 2        |           |           | 31125     | Cuitimad    | 9.021    | 1230        | 3.07     | 0.00000    |                 |              |                      |     |   |
| 41       |           |           | 11.0      | Curtimat    |          | 1.3-00      | 2.05     | 13+405     |                 |              |                      |     |   |
| <b>H</b> |           |           | 10.00     | Continued   |          | 3.8+985     | 1.25     | 7.44-005   |                 |              |                      |     |   |
| 2        |           |           | 1010      | Cardonad    |          | 44+005      | 2.52     | 14+105     |                 |              |                      |     |   |
| 12       |           |           | 101       | Canformal   |          | 12-05       | 4.78     | 1.3+-005   |                 |              |                      |     |   |
|          |           |           |           |             |          |             |          |            |                 |              |                      |     |   |
| 87       |           |           | 1019      | Carfornell  |          | 0.00034     | 1.21     | 1000034    |                 |              |                      | 1   |   |
| 41       |           |           | 1120      | Continued   |          | 1.2e-085    | 3.25     | 5.2×-005   |                 |              |                      | -   |   |
| 5        |           |           | 10787     | Cartimed    |          | 1.0018      |          | 0.00918    |                 |              |                      | -   |   |
|          |           |           | 1195      | Continued   |          | A.Se-005    | 3.85     | 4.54-005   |                 |              |                      | 1   |   |
| 2        |           |           | 1,08      | Conformati  |          | 3.76-005    | 1.78     | 5.7+005    |                 |              |                      |     |   |
| 12       |           |           | 1,719     | Conformal   |          | 0.00045     | 2.76     | 0.00980    |                 |              |                      |     |   |
| 14       |           |           | 1223      | Continued   |          | 0.00012     | 2.31     | 0.00012    |                 |              |                      |     |   |
| 34       |           |           | 1234      | Cardomad    |          | 22e-005     | 1.38     | 2.3e-005   |                 |              |                      | 3   |   |
| 25       | _         |           | 3,040     | Continued   | 111111   | 4.6e-903    | 5.28     | 88+005     |                 |              |                      | 4.1 | 8 |
| 5        |           |           |           |             | 1000     |             |          | 7.04-000-  |                 |              |                      | -   |   |
|          |           |           | 1241      | Continued   |          | Film-Stift. | 141      |            |                 |              |                      |     |   |
| 1        |           |           | 1248      | Continuat   |          | 8.89005     | 4.50     | 0.00025    |                 |              |                      |     |   |
|          |           |           | 1.298     | Continued   |          | 3.5e-000    | 3,28     | 2.5x:005   |                 |              |                      |     |   |
| 10       |           |           | 1.119     | Confirmati  |          | 4.04-005    | 2.62     | 4.0x/005   |                 |              |                      |     |   |
| 45       |           |           | 1112      | Certimat    |          | 3.24-005    | 442      | 3.24-005   |                 |              |                      |     |   |
| 12       |           |           | 1338      | Continued   |          | 8.00042     | 2.11     | 0.00042    |                 |              |                      |     |   |
| 40       |           |           | 185       | Conformati  |          | 100045      | 4.28     | 0.00043    |                 |              |                      |     |   |
| H .      |           |           | 1386      | Confirmed   |          | 15+98       | -178     | 2.5x-005   |                 |              |                      |     |   |
| 10       |           |           | 2,284     | Confirmed   |          | 6.09630     | -2.78    | 0.00020    |                 |              |                      |     |   |
| 14.      |           |           | 3428      | Cardonal    |          | 8.0e-085    | -115     | 8.2w (875) |                 |              |                      | 3   |   |
| 12       |           |           | 3445      | Cardimat    |          | 15e-885     | -2.11    | 2.3a.005   |                 |              |                      | 1   |   |
| 14       |           |           | 3403      | Carfornal   | 1782     | 0.09076     | -2.22    | 0.30078    |                 |              |                      | 1   |   |
| 44       |           |           | 3484      | Continual   | 32.02    | 32e-088     | -5.28    | 3.3x-005   |                 |              |                      | 1   |   |
| 20       |           |           | 1572      | Continued   | 52 825   | 1.2x-085    | -2.34    | 1.2x-005   |                 |              |                      | 1   |   |
| 11       |           |           | 3379      | Canfirmal   | 1202     | 5.74-005    | -1.25    | 3.74-005   |                 |              |                      | 1   |   |
| 14       |           |           | 3111      | Continued   |          | 8.00028     | 111      | 9,00113    |                 |              |                      | 1   |   |
|          |           |           |           |             |          | -           |          |            |                 |              |                      |     |   |

Expert: where the T-test value, average ratio value, one D ANOVA value, these all we can see here in the table view. So the 4 views at a time

#### (Refer Slide Time 10:14)

| Gel 03 Cys Control and  | Get B1 Cyh Trostedgol  | has   |
|---|--|---|
| Gal D2 Cy5 Costrol and  | Get 02 Cyst Product and  | Distain 120 Masserier 1261 Pro.                           |
| Gel 03 Cel Control pel  | Gel 03 (55 Treateday)  |   |
| Gal D4 Cys Central and  | Gel 06 (2) Freehold and  | LM No 130 Marin Ro 130 Pic.<br>Graph View - Henler No. 1. |
| Protein Table T first and An Ratin: Treated / Control<br>Page Data Protection Masses Internet (Control<br>4) WR Conference (CO) (C)<br>41 USS Conference (C) (C)<br>41 USS Conference (C) (C) | 1         Sor. Tester.         3 48425         2 4882564. Content         2 4882564. Content |   |

Expert: to see This is the four views we can see here. So after this, we can filter them according to our interest.

(Refer Slide Time 10:23)

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| 4 Put Puter 10 Uniter 100  | E  | -1                    |  |
| 42 303 044   |  |                       | 1 1 2 3 2 3  |
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|  |  | Ten Cont Mark         | Facus Frances Table MLAN   |
|  |  |                       | Pacas Protein Table NEAL   |

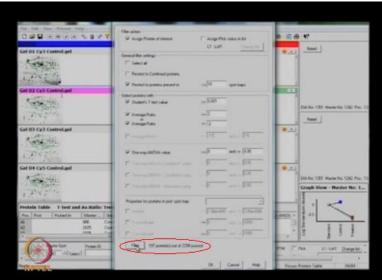
Expert: So select few parameters which are available

(Refer Slide Time 10:28)

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| L'ENE .                                | CR Annual Ann                            |                     | 1 4 4                                |
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| 42 3025 Com                            | Frank                                    |                     |                                      |
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|  |  | [ ] Canel Har ]     | Ferrar Proton Laine MOAN             |
|  |  |                     | Press Press rate State               |

Expert: restrict to 10 gels which are spot maps, which are present. A particular protein should be there and student T-test as well as average ratio, then one way ANOVA value and filter it

(Refer Slide Time 10:47)



Expert: So there are 2299 spots are available in these all gels but 107 proteins only passed all these parameters. So these parameters we can select as a protein of interest and

|  | Film action<br>W Accep Proster of Innear  | Canage Print manager            | 204                                   |
|--|---|---------------------------------|---------------------------------------|
| Gel 03 Cy3 Control.pel   | Interference  |                                 | Anat_                                 |
| - Range Tett   | C Selected  |                                 |                                       |
| · A star starter   | C Renard Contract process   |                                 |                                       |
| and the state  | and the second se | The second second second second |                                       |
| Get 02 Cys Centrol pel   | W Rachell's primer prime in   | Selfit spinner                  |                                       |
|  | Tokat jadam self  | 1. 1. 10                        |                                       |
| - Martin   | P Suderh Tract value  |                                 | DATE THE Made To LAP Port             |
| A DESCRIPTION  | P Amage Sale  |                                 |                                       |
| All and the second seco | P Arrespe Take  | 14.52                           |                                       |
| Gel 03 Cy3 CoetroLard  | Constant  | - 113 - 12                      | ·                                     |
| - Martine  | A Competence  |                                 |                                       |
| The parts  | W Design MDD rate   | 1.5 min 2.8                     |                                       |
| There  |   |                                 |                                       |
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| - Altered  |   |                                 |                                       |
| No. of Concession, Name  | 1   | DR Canoni Hub                   | Forces Frates Takes Name              |

(Refer Slide Time 11:09)

Expert: assign pick list so that these proteins can be saved in a file. This file can be given furtherly to spot picker.

(Refer Slide Time 11:21)

|   | Transfer and the second se  | 1                          |
|---|---|----------------------------|
| Gel D1 Cys Centrolael   | Gel B1 Ces Traund gel   | 1                          |
| Gel 03 Cys Castrol gel  | Gal 92 Cy3 Treated gal 22   | To be Million and State    |
| Gal 03 Cy3 Cantrolgad   | Gel 83 Cys Treatest gel   |                            |
| Gal Of Cys Control and  | Gel 64 Cp3 Treated gel  | Cough View - Hanlar No. 1. |
| Protein Table         T lest and As Ratio: Trouted / Control           Pes         Point         Pointers         Nature         Nature           Pes         Point         Pointers         Nature         Nature         Nature           Pes         Point         Pointers         Nature         Nature         Nature         Nature         Nature           Point         Pointers         Pointers         Nature         Nature | 1 mm         in         fame         1.4902.         2.482296 Counts.         2.000296 Counts. |                            |

Expert: These are all the things we are enable to identify in BVA. This is very user-friendly. There is no much more manual interference, Ok. This is what this helps you to analyze your DIGE gel.

(Refer Slide Time 11:39)

|  |                                 | 13 540                       |                       |  |
|--|---------------------------------|------------------------------|-----------------------|--|
| Gel 01.Cy3 Control.gel   | Court No 1999                   |                              |                       |  |
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| APPER  |                                 |                              |                       |  |
| Gel 02 CyS Control and   | C Recently polyage present an   | (a)) quinas                  | -                     | and the second   |
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| and the second s | P Inden Tren use                | 10 R.M.                      |                       | ANALYSIN Many No. 1201 Fig. 1  |
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| NPTEL  |                                 | and the second second second | and the second second |  |

(Refer Slide Time 11:45)



Professor: So, can you elaborate on what is EDA or Extended Data Analysis? What it can do which we are unable to do in BVA? So there is layers here, right, one is DIA followed by BVA...

Expert: Yeah

Professor: And then ultimately EDA.

Expert: Exactly. Basically what we can do here is we can compare two BVAs together there exactly. Here we are talking about a particular disease or a particular set of data only. There we can analyze different BVAs together in EDA, there you can get majorly Differential Expression again you will get as well as PCA and Discriminant Analysis, these kinds of statistical data you will get in EDA. Very shortly, I will just show briefly....

Professor: If I understood correctly, probably the statistical parameter will be more stringent over there in EDA...

Expert: Yes

Professor: We can have some better biological significant information...

Expert: Exactly

Professor: From dataset

Expert: Exactly.

Professor: Because in lot of clinical data or different types of treatment, people like to do several gels and lot of treatment.

Expert: Yes

Professor: So your number of samples to be analyzed is very large.

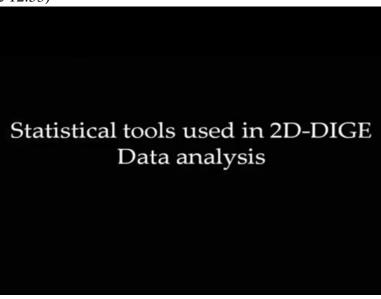
Expert: Exactly

Professor: And really obtaining the meaningful information is the real challenge.

Expert: Exactly

Professor: In all the protein analysis. So I would like to see now EDA.

(Refer Slide Time 12:53)



(Refer Slide Time 13:03)

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| Fotier comments        | 24 799 799   |                    |            | 1997          |
| free servest           | <b>33</b> 840 810  | 41                 |            | 14.2          |
|                        |  | 111                |            | 792           |
|                        | <b>33</b> 840 810  | 122                |            | 942.<br>***   |

Expert: Thing which we can see here, Differential Expression Analysis in which you can see differences in

## (Refer Slide Time 13:06)

| Setus Calculations                                    | - Kasajila Interpretation<br>Alpha  | 2 December Analysis  |                       |                |
|---|---|--|-----------------------|----------------|
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| Alber Set   | Average ratio.<br>Reader(1) 1 North   | 84<br>636<br>635   |                       |                |
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|   | 28 788 788  |  |                       | 728            |
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| 13  |   |  |                       | 762            |
|   | 28 812 812  | 100  |                       |                |
|   | P# 852 852  |  |                       |                |

Expert: between control as well as two experimental data

(Refer Slide Time 13:11)

|   | - Rasalla Interpretation<br>Mijam 10 Annugal Companyon Analysis 🖉 Yattaon Analysi  | a Starouran Analys |                     |              |
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|   | prettern ELA   |                    |                     | 2.20         |
| Hitter SAL.   |  | 84                 |                     |              |
| View set in sets  | Average ratio  | 1.06               |                     |              |
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Expert: This is the different treatments which is given Here you can see how the particular protein is ...

(Refer Slide Time 13:14)

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| -  | 79 758 .718                                  | 14                            |                | 787           |
| Contraction of the local distance of the loc | 26 789 799                                   | -                             |                | 759           |
| 1  | <b>79</b> HER AVD                            | 11                            |                | 780/          |
| A Starter  | 78 813 812                                   | 100                           |                | 742           |
| 1  |  |                               |                |               |
| 1000   | upo 814 818                                  |                               |                |               |

Expert: expressing throughout these control as well as this Pf and Pb, this kind of things and you can see this kind of data for each and individual protein here. So that from here, you can see which one is your interest and which is not...

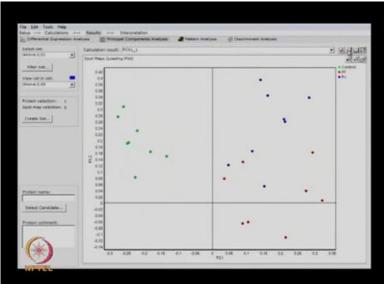
(Refer Slide Time 13:30)

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Professor: you are actually...You are actually analyzing the data spot wise now, spot by spot you are looking...

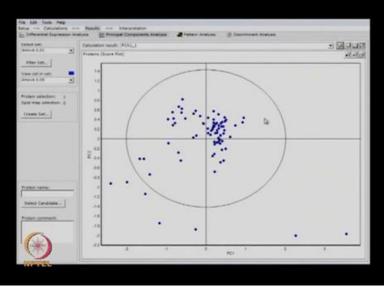
Expert: Exactly here the spot by spot which we are seeing, the number of ...even the index number shows there is a master gel, from the master gel you can see exactly this number. This is what which we are seeing here for each and individual spot, here we can see the results as well as

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Expert: we can see the Principal Component Analysis of this data.

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Expert: Here there are almost 89 proteins, out of these 89 proteins, you can see, inner the circle, there are proteins, some proteins are present, and out, out layers are there. The inner circle, they are similarly, especially if I can say 95% statistically significant is there, and out layers which you can see are exactly, these can be some non-reproducible spots or else what the major thing is, these are all very highly up-regulated or highly down-regulated. So this can be worked as the marker also. Then we have to go back to our BVA data and we can check the protein, how exact it is regulating. Then we can identify the protein and we can use for further analysis.

Professor: So this is a powerful statistical parameter

Expert: Exactly

Professor: by using which you can identify some outlets

Expert: Exactly

Professor: which could be the potential discriminator between the control...?

Expert: Control and treatment.

Professor: And once you identify those proteins, you can go back to your original data for the BVA

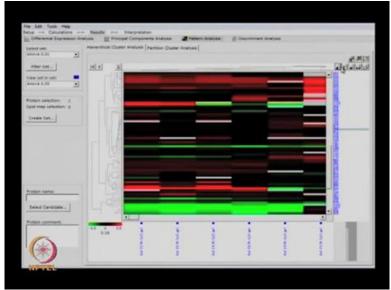
Expert: BVA

Professor: And data analysis is done

Expert: Exactly

Professor: This is very interesting.

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Expert: And next pattern analysis is we can see the whole proteomes and how there are difference from each other...

Professor: So this is the heat map

Expert: This is the heat map of the total 82 proteins which are taking into consideration, then how, in which area they are up-regulated, we can see the blue area,

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| File Edit Tank Help<br>Tether Calculations<br>Differential Expression Ana | Assults | 1999     | etalium |               |   |   |   | _   |        |
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Expert: sorry the green area exactly we can see is completely down-regulated area



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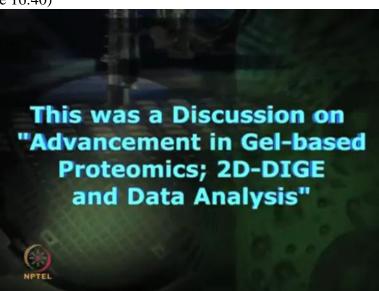
Expert: and the red color portion which you can see, that is the up-regulated portion and the remaining black color which you can see, those proteins are similarly regulated. This is what you can see here. This kind of data will help you to represent your complete, whole analysis.

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Professor: Doctor Srinivas, it was very useful to have you here and to get an overview of DIGE technology, how people can use this type of software and analyze their data by using DIA, BVA and EDA and although there was not enough time but you gave a very good demonstration in a very short time to give a glimpse of the processes involved in doing this analysis as well as how different types of statistical parameters can be applied to get some very powerful statistical information from our biological data. So thank you very much for coming here and giving this very good introduction about DIGE technology

Professor - expert conversation ends



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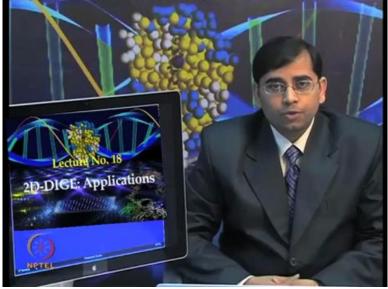
So I hope our discussion with Doctor Srinivas was useful and now you can perform these analyses by using specialized software and obtain some very useful biological information from your data set. Probably you must appreciate there are lots of meticulous steps involved in performing these experiments but at the end this provides very useful, quantitative, multiplexing approach to separate proteins and to analyses different types of variations.

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# Points to ponder

- BVA is used for analyzing the Cy2, Cy3 and Cy5 gels of more than one biological replicate
- One of these Cy2 gel is selected as Master gel and other gels are matched against this gel
- Four modes of BVA: Spot Map Mode, Match Mode, Protein Mode and Appearance Mode
- BVA gives 3-D view, graph view of each spot

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I hope at the end of this module and lecture you will be able to perform gel-based proteomics experiment. But please keep in mind these protocols and methods are only giving you a feel for performing these experiments.

Each experiment, each sample type, each biological question brings its own unique challenges and depending upon those conditions and your sample type you need to optimize

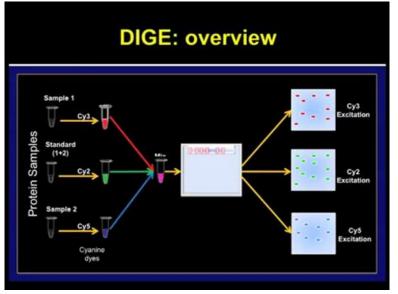
these methods. There is no one technology which can answer all of your questions but it is good idea for you to know; what are different methods that are available for you to use.

So I hope by taking this module on gel-based proteomics, now you are familiar with different types of gel-based techniques. These are only few, there are many other methods as well available but these are the most commonly used methods which people are applying in the field of proteomics.

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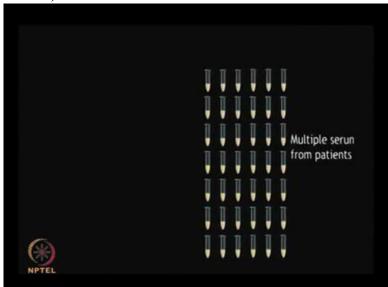
Say among 2DE and DIGE, which of these two techniques will be better to

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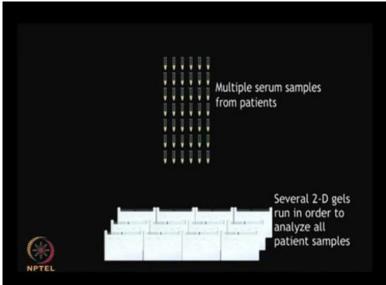
separate serum protein samples obtained from large number of patients in a clinical trial?

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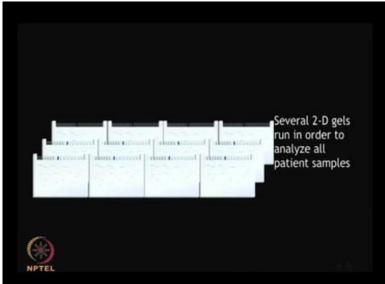
If I have multiple serum samples

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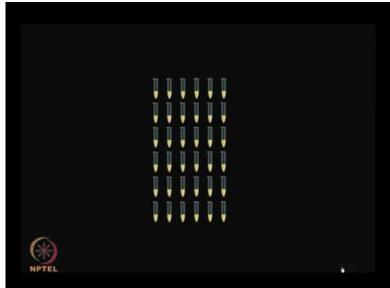
from patients, two-dimensional electrophoresis although a very useful technique,

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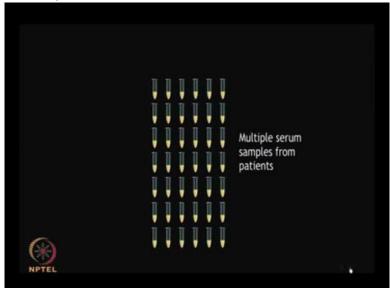
but it may not be the best option in this case to analyze serum proteins from large number of patients.

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In this case DIGE will be extremely valuable tool for analysis of large number of samples

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simultaneously without having to overcome

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| Control<br>samples |   |   |   |   |   |  |  |
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| Test<br>samples    |   |   |   |   |   |  |  |
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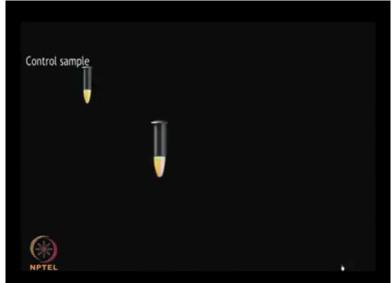
the problem of

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| Control<br>samples |  |  |  | Internal |  |
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| Test<br>samples    |  |  |  | samples  |  |

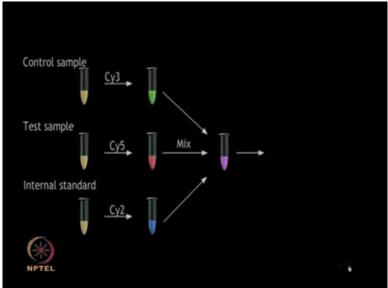
Gel-to-gel variations

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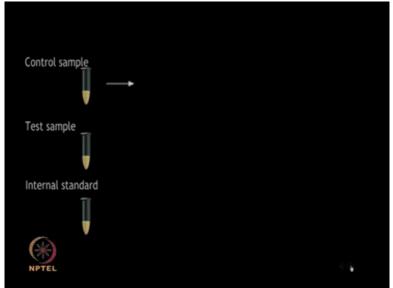
In DIGE gels the control and the samples can be differentially labeled by using the cyanine dyes and then run on the single gel.

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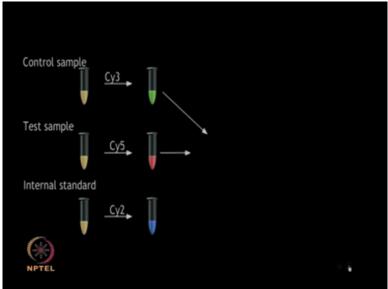
The pooled internal standard

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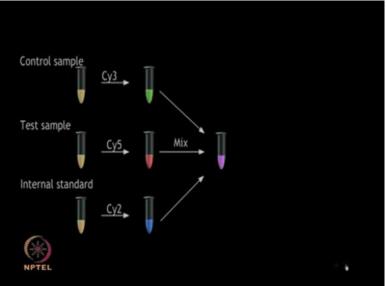
for DIGE

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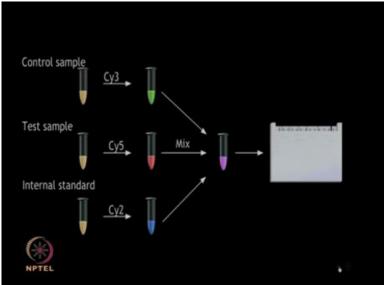
is prepared

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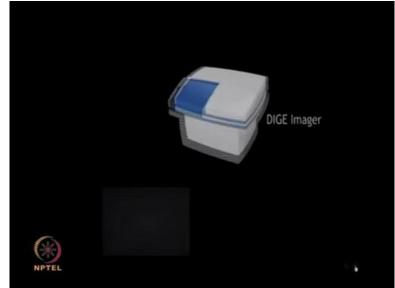


by mixing

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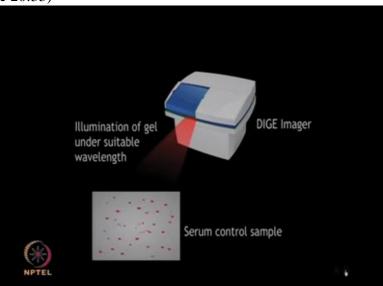


equal amounts of all the samples that need to run on the gel and this prevents the problem of gel to gel variations.

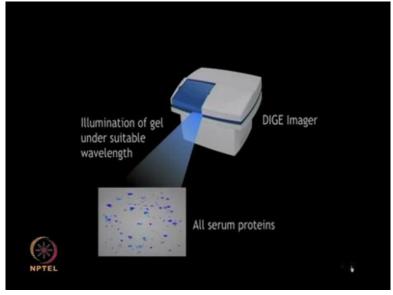


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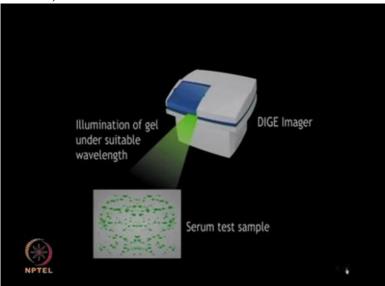
From the same gel, three different images can be obtained for Cy2,



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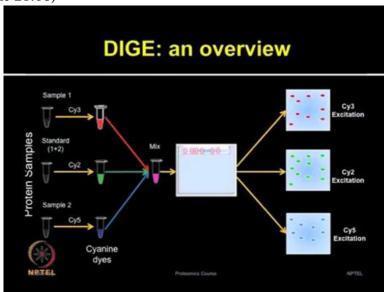
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and Cy5, therefore there would be no reproducibility issue and various artifacts can be eliminated for the clinical or the large number of sample masses.

The main aim for development of Difference in Gel electrophoresis was to overcome the inherently poor reproducibility of conventional two-dimensional electrophoresis.



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The DIGE is quite sensitive technique with less than one femtomolar of proteins which can be deducted, and it can enable the linear detection of very broad dynamic range of the proteins. So, as you can see in this slide the protein samples directly have control, and treatment those are labeled with two different dyes Cy3 and Cy5, but a small aliquot of both of these samples is mixed together to make a internal pool.

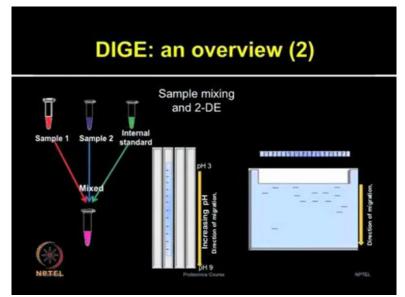
That internal pool is labeled with third dye Cy2.All these three protein samples are mixed together in one tube, which contains both control treatment, as well as the reference spots from the internal pool. All these protein mixture are separated in the first dimension on the same strip, and then the same gel can be scanned with the three different wavelengths to obtain the images for the Cy2, Cy3 and Cy5.

> DIGE: an overview (2) Sample mixing and 2-DE

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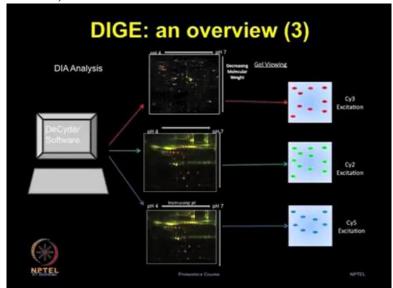
So, in the conventional 2 dimensional electrophoresis the gel-to-gel variations which come from the acrylamide polymerization, electrical pH and thermal fluctuations in different gels that can be overcome in the DIGE gels, because all the protein separation is going to happen on the same gel. So, all those artifacts can be minimized by using DIGE approach.

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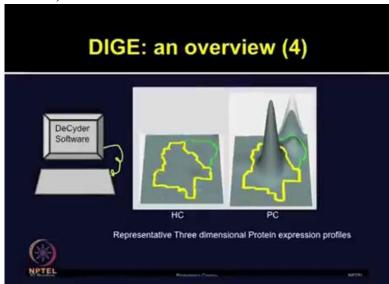
In the slide it is shown that the three samples are mixed, and then isoelectric focusing is performed from the pooled sample, and then this strip is placed on the SDS page gel for the protein separation in the second dimension.

So, overall DIGE provides very uniform staining from gel to gel, and shows high sensitivity and linear dynamic range of detection for the expression profiling of complex biological samples. See, for aim is to resolve thousands of proteins, and cover comprehensive proteome coverage, then DIGE is a very good platform.



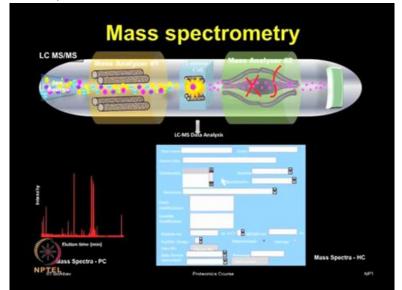
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Especially, if you want to do the comparative or differential proteomic analysis, because your gel to gel variations and other variations will be minimized, and DIGE will provide the very high sensitivity



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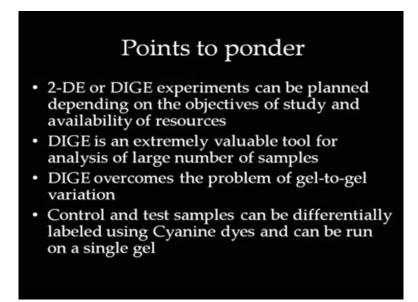
So, once you have run these gels, now from the same gel the images can be obtained, three images of your control and the treatment, and these can analyzed from different software, such as DeCyder software, and then by looking at 3-dimensional views and the statistical data, then these proteins can be considered as interesting for further investigations.



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Once the spots are analyzed and excised from the gels, then the same tradition you have to follow, you can use any of the mass spectrometry platforms, and then obtain the MS spectra for further analysis using different type of bioinformatics tools, such as Mascot.

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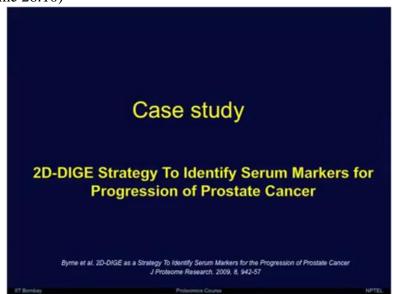


So, overall the DIGE method is far more superior in terms of the reproducibility as compared to the conventional two-dimensional electrophoresis, and for the quantitative accuracy.

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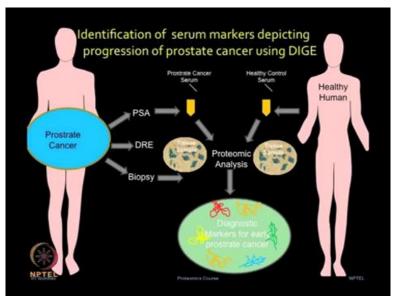
Therefore, applications of 2D DIGE can be found in virtually all major biological research areas. If you see the recent publication, you will appreciate there are several papers on each of the biological system for different, different type of applications, whether its cell signaling, looking at developmental biology, looking at plant proteomic analysis, neurosciences, clinical studies, different type of diseases including cancer, you will find there are hundreds of publications available, which have employed the power of 2D DIGE technique.



Let us talk now a new case study, case study 3 on 2D-DIGE as a strategy to identify serum markers for the progression of prostate cancer. Study by Byme et al, published in 2009. So, this study authors aimed for identification of serum markers by depicting the progression of prostate cancer, by using difference in gel electrophoresis technique.

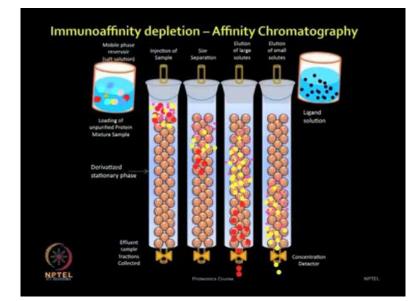
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So, prostate cancer is recognized as a significant problem in older male population. The prostate cancer screening relies heavily upon testing for the higher level of Prostate Specific Antigen, also known as PSA within the peripheral circulations.

So, PSA is very sensitive marker, but the lot of discussion on reliability and specificity of PSA for the prostate cancer. Reason being that the level of PSA is also high in benign prostatic hyperplasia or prostatitis. So therefore, there is lot of discussion whether one should rely on only PSA for detection of the prostate cancer. So, this study aims to identify some new markers in the prostate cancer by studying the serum proteome analysis.

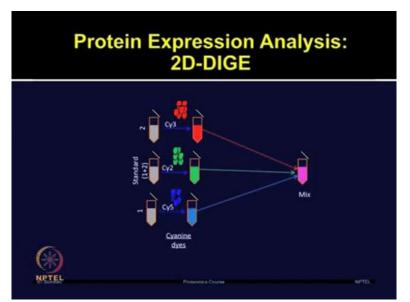


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As you are aware, and in fact we have discussed the protein preparation from the serum earlier, so each of the biological sample poses lot of technical challenges, and serum is one among them where presence of highly abundant proteins, such as albumin and immunoglobulin, they result into the masking of low abundant proteins.

So, to eliminate those high abundant proteins, authors used multiple affinity removal system from the Agilent technologies, and they removed most highly abundant proteins from the serum sample including albumin IGG antitrypsin IGA transferrin and haptoglobins. So, after the abundant proteins were depleted from the serum sample, then authors moved for the protein extraction and further analysis.

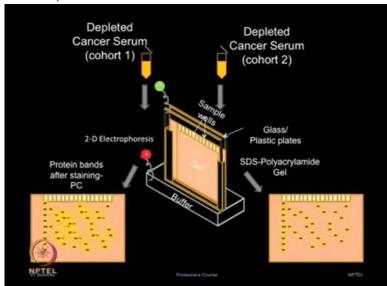
So, the differential proteomic analysis was performed in the two different cohorts of histologically confirmed prostate cancer, with different grades of the disease.



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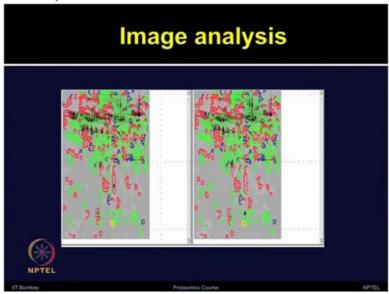
They used the patients with two different grading system based on Gleason grading. So, the Gleason grading system that is used to help and evaluate the prognosis of men with the prostate cancer The depleted serum samples obtained from then patients with Gleason score 5 and Gleason score 7 were used for comparison and further analysis.

As you can see in the slide these samples were first labeled with the Cy3, Cy5 and also the internal reference pools were made which were labeled with the Cy2 dyes. These samples were then further mixed, the depleted cancer serum from first cohort of Gleason score 5,



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and second cohort of Gleason score7, those were mixed, separated in the first dimension and followed by protein separation in the second dimension.



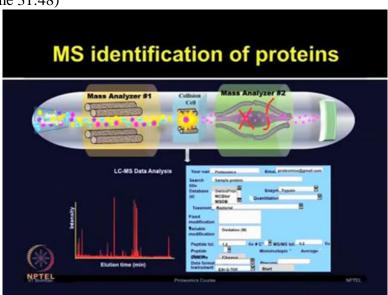
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Now, when authors analyzed these DIGE images, they found at 63 protein spots were differentially expressed between the Gleason score 5 and 7 cohorts, and 13 of these proteins were statistically significant among these two populations.

So, as you know analysis of these gels is always challenging, especially if you are looking at the conventional 2D gels, where you have separate gels obtained from each of this groups. But, analysis in the DIGE gel is more automated, so if you remember our previous discussion in the DIGE gel analysis.

This analysis is more automated and more straightforward, but still we have to through individual spots, and we have look for the how real, how significant those changes are, and you have to look at the 3D views of those spots to ensure that it is the reproducible among various control and treatment groups.

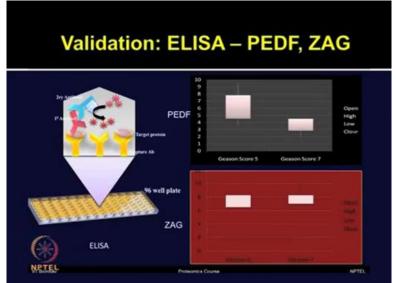
There are different levels of analysis performed which we have talked earlier, but this just shows the final output that 63 spots after all the analysis steps were considered significant



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After2D-DIGE gel image analysis, authors excise those spots, and subjected for the mass spectrometry identification of proteins, so the proteins excised from the gels were analyzed by using Finnigan LTQ mass spectrometer, and data from these MS/MS experiments were analyzed by using Bioworks Browser by using SEQUEST program.

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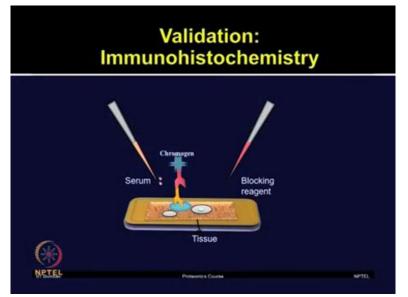


For validation, authors employed various techniques. including Western Blots, enzyme linked immunosorbent assay, ELISA and also immunohistochemistry, Pigment Epithelium-Derived Factor PEDF and Zinc Alpha 2-Glycoprotein often known as ZAG; those proteins were further validated by the ELISA technique.

So, the PEDF levels were quantified by using ELISA kit, and results demonstrated as you can see in the slide that the statistically significant decrease in the PEDF in the Gleason score 7 depleted serum groups.

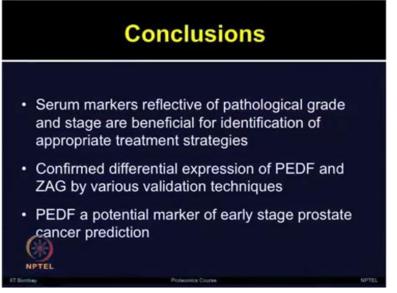
Whereas, the results for Zinc Alpha 2-Glycoprotein ELISA analysis, which is shown in the red in the bottom panel that indicated 1.4 fold increase in the Zinc Alpha 2-Glycoprotein absorbance in the Gleason score 7 group. So, these studies, this ELISA validation confirm their findings from the2D-DIGE experiments

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Authors also employed immunohistochemistry, or IHCs for validating the pigment epithelium derived factor PEDF and zinc alpha 2 glycoprotein, so that they are very confident that the proteins which they have identified from the proteomic profiling, those are real, and they also tested those on the independent tissue samples.

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So, from this paper the major conclusions were that serum markers, which are reflective of the pathological grade and stage could be beneficial for the identification of appropriate treatment strategies.

Authors confirmed that differential expression of PEDF and ZAG can be performed by using various techniques, such as Western Blot, ELISA and immune histochemistry.

Based on the studies and the follow up experiments, they concluded that PEDF could be a potential marker of early stage prostate cancer prediction. However, more studies and follow up required on the large number of patients before it can be established a good biomarker

You may appreciate that there is lot of power of these techniques and these can be employed for any biological application. You pick an application of your choice and I am sure you will be able to answer those by employing 2D or 2D DIGE techniques. Thank you.

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## Summary

- 2D-DIGE analysis was demonstrated
- DIA, BVA modules were discussed
- An overview of 2D-DIGE was discussed
- Case study of 2D-DIGE was discussed