TOTALLAB

SameSpots

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SameSpots Case Study

Background Information

"We are using proteomics, 2-dimensional gel electrophoresis to identify protein changes in heart disease to further our understanding of the mechanisms and molecular basis of biological processes involved in a trial fibrillation. This will help us identify new biomarkers that can be exploited as diagnostic/ prognostic reagents and/or as therapeutic targets. Recently the statistical power of SameSpots allowed us to very quickly identify and reject an outlying sample during a control vs. diseased experiment. Without this benefit we could have easily encountered a number of problems further along the analysis and our statistics would not have been as robust."

Our 2D experiment design

"We were analysing the proteome of left atrial cardiomyocytes, comparing control vs. disease samples using large format 2-D gels, approx. 24 x 24 cm. These were stained using DIGE – Cy2, Cy3 and Cy5; followed by silver staining for protein visualisation and spot picking. In this experiment we ran two technical replicates per sample, using a Cy3/Cy5 dye swap, with five biological replicates in each group."

The challenges we face in our 2D image analysis

"Before SameSpots, analysis of 2D images was time consuming, despite having good images. The software that was previously used was not particularly user friendly, and a lot of user intervention was required."

How SameSpots helps us to meet the challenges

"The main difference is the speed at which we can analyze our gels. Using SameSpots, we are able to analyze our gels in a much shorter time frame, with very little manual intervention required. Initial analysis that could take days before can now be achieved within a few hours, obviously depending on the number of gels. The results we are obtaining are extremely robust and reproducible. The matching is excellent, and hardly any manual spot editing is required. Also, the inbuilt statistics is user friendly, giving both q values, which are extremely useful to have in addition to the p values, along with the power value. The workflow is straightforward and logical, and visually appealing."

How the statisical power of SameSpots helps us

"Using the inbuilt PCA analysis, we found that there were two images that were definite outliers in one of the groups that we were comparing." See figure 1 below.

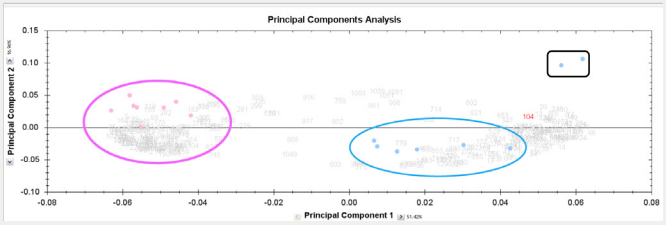

Figure 1: PCA plot for the two groups in the experiment. Control (pink oval) and diseased (blue oval). It is clear that there are two outliers in the diseased group (black square) These were identified as the Cy3 and Cy5 images from the same sample. Further investigation revealed this sample had an issue, hidden within the meta-data, which would confound results. With this additional knowledge it could be confidently discarded from the experiment.

"Following this, we went back to check which samples these were, and found that they were the same sample labelled with Cy3 and Cy5. This lead us to believe that this was a true finding, and further investigations as to why this sample was an outlier were investigated. On this particular sample, it was noted that there was some missing characteristic data, so we relayed this back to our collaborators to see if they could shed any light on this. The outcome was that indeed this sample was behaving in a different way to the other samples in this experiment, hence why I was missing some data, also explaining why this particular sample was an outlier. For this reason, this sample was omitted from further analysis.

What advice would you give to a proteomics scientist struggling with analysis problems?

"I would advise anyone performing 2-D gel analysis to give SameSpots a trial, as I am sure you will be converted to this easy to use, hassle-free software."