

Measurements of Reliability and Completeness in Image Analysis of Two-Dimensional Electrophoresis Gels

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In two-dimensional gel electrophoresis (2DGE) experiments, the achieved correctness in the image analysis plays a crucial part in maximizing the number of true protein changes found in the experiment as well as minimizing the error rate among the results.

In this study, we measured the correctness of different aspects of the image analysis in order to investigate if there is a way to predict the reliability and completeness of the image analysis results. As relevant measures we used the present proportion of noise in the form of false positives as well as the number of verified, statistically significant changes in protein expression that are found, given that these are the most important factors in the desired outcome of most 2DGE experiments.

We had access to a large number of gel image analyses performed by the Ludesi Analysis Center as well as by a variety of organizations using analysis software such as PDQuest, DeCyder, Progenesis and Progenesis with SameSpots. A novel method of manually evaluating the correctness of different aspects of the analyses, such as spot detection, spot segmentation and spot matching, was devised. In addition, we calculated the mean coefficient of variation (mean CV) of the spot volumes in all the analyses.

We will present resulting data to show that a combined measurement of the spot detection correctness and spot segmentation and matching correctness display a great degree of correlation with the number of statistically significant protein regulations that are found, as well an inverse correlation with the fraction of errors among these regulations. In contrast, mean CV is much less useful when trying to predict the reliability and completeness of the analysis.

The results indicate that a measurement based on rigorous evaluation of the correctness of spot detection, spot segmentation and spot matching, which we have termed *Combined Correctness (CC)*, provides a good indication of the reliability and completeness of the results from 2D gel image analysis.