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Editorial

Quality control for SELDI analysis

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Surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI TOF-MS) studies have claimed significant success in identifying biomarkers with higher sensitivity and specificity for detecting disease than currently available diagnostic methods (1–3). While these studies demonstrated great promise, the quality of SELDI spectra was not evaluated systematically until two years ago. In 2003, the analysis of a published dataset demonstrated the role experimental bias can have in spectra quality (4). Several articles have since been published that investigate the reliability of SELDI spectra output. The article by Manual Aivado and colleagues (5) published in this issue of the journal should be a welcome addition to this important area of research.

This article has demonstrated several important analytical parameters which may affect the experimental outcome of a SELDI study. First, the authors were able to show that automation improves spectra quality by reducing variation. Second, they provided additional evidence that peaks with a low signal to noise ratio (SNR $\leq$ 2) should be excluded from analysis. By increasing the SNRs from 2 to 5, the number of peaks was reduced as well as the peaks' coefficients of variation (CV). Third, this article discussed a number of issues relating to SELDI reproducibility and demonstrated that standardization of analytical parameters was an important step toward better performance.

In conducting SELDI experiments and performing data analysis, one should be aware of other important issues. First, using cluster analysis as a means of selecting an optimal number of replicates might be useful. However, many researchers are now spotting their samples in replicates. A comparison of different methods for identifying spectra replicate inconsistencies would also be informative. Second, while this study showed that freeze-thawing up to 5 cycles did not deteriorate the CVs significantly, the analysis methods used here may not be sensitive enough to detect a set of proteins which decay at a faster rate than the highly abundant proteins. Third, their evaluation of SELDI spectra quality seven months after the initial analysis is critical to evaluating spectra reproducibility. It would also be interesting to cluster all of the data from the two time points to determine if the sample identity or the time of analysis were more influential in defining the clusters.

In the current literature, several articles have demonstrated that it is important to optimize SELDI experimental parameters in order to achieve good quality spectra. Therefore, one should define SELDI quality assurance and quality control (QA/QC). One of the first articles published used multi-factorial study design to identify optimal sample preparation and chip spotting procedures (6). However, unlike the article published here (5), a robot was not used, and their statistical analysis did not include cluster analysis. Another study was published just months later which used bioinformatics analysis to identify chips with questionable spectra quality (7). While this method was rather expensive in that one quarter of the chips' spots were dedicated to assessing chip quality, it highlighted several issues which had heretofore been unexplored; including bias associated with chip and day of processing, and the need for a statistic to measure how different a spectrum is from other spectra observed on the same sample. And, finally, two multi-institutional SELDI studies will be published this year. In both studies, a set of standard protocols were used for sample processing, chip spotting, and spectra collection. The spectra obtained using these protocols were compared across multiple institutions. With these rigorous standard protocols, both studies obtained very consistent spectra. One of these was supported by the Early Detection Research Network and was published in January of this year (8). The second study was completed for the Human Proteome Organization and will be published this summer in *Proteomics*.

The issues described here are not unique to SELDI analysis; they are shared by other large-scale proteomics expression analysis techniques. This research indicates that QC combined with standard operating procedures allows SELDI users to produce high quality spectra. The study by Manual Aivado and colleagues has provided additional evidence for improving reproducibility and standardization among SELDI experiments while highlighting factors that contribute to variability. Such research can only improve the quality of SELDI applications.

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