

Impact of 2-Dimensional Electrophoresis Image Analysis on the Output from Proteomics Experiments

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Abstract

Image analysis of 2-dimensional electrophoresis gels, consisting of spot detection, matching and segmentation for all gels in an experiment, has proven to be a significant bottleneck in both rate and quality of proteomic analyses. Computer-based automated analyses of gel images tend to require a significant amount of manual correction. These corrections introduce variability and bias into the analysis of the images. All automated image analyses on the other hand have a propensity to generate errors in spot detection, matching and segmentation. The bioinformatics company Ludesi has devised a unique pay-per-image analysis method which bypasses user variability and errors with a professional analysis of spot detection, segmentation and matching of the protein spots for all included gels, using an all-to-all gel comparison system. Three independent experiments (n=9, n=10, n=6) were performed on silver-stained 24 cm, 150 µg protein human serum gels and selected Cy5-labeled fluorescent gels, using the Ludesi Image Analysis method, Progenesis v2005 (with and without manual correction), PG240 with SameSpots warped with TT900, and DeCyder 5.01. Comparisons were made for number of correct protein regulations detected (experiments n=9 and n=10 only) and correctness of spot detection, spot matching and spot segmentation respectively. Ludesi's Image Analysis method provided an overall higher correctness, resulting in a significant increase in the ability to find correct protein regulations compared to Progenesis and PG240 with SameSpots.

Methods

2D Electrophoresis:

Silver-stained Gels:

- 150 µg of albumin and IgG depleted serum focused on 24 cm pH 4-7 IPG strips (Amersham). Second dimension resolved using multicast tris-glycine or bis-tris gels on a PROTEAN plus dodeca cell horizontal system.

- Silver-staining based on Schevchenko *et al.* protocol

- 150 dpi 16-bit greyscale images were acquired on UMAX powerlook III scanner.

Cy-labeled gels

- 5 µg depleted serum samples labeled with Cy3 or Cy5 added to 5µg of Cy2 labeled pooled standard, focused on 7cm pH 4-7 strips and resolved on bis-tris gels.

- Fluorescent images acquired on a typhoon 9400 laser imager at 50 microns and voltage settings of 580V (Cy3, Cy5) and 590V (Cy2) .

Image Analysis:

Progenesis Analyses:

- Three projects two silver stained (n=9, n=10) and one Cy-labeled (n=6)

Silver-stained Automated Analysis:

- Single stain, auto-selected reference gel, spots detected-default parameters, Progenesis background subtraction, warp and matched to reference combined warping and matching, unmatched spots added to reference and normalized to total spot volume.

Manual analysis:

- Automated Progenesis silver-stain projects manually corrected for spot detection (boundary corrections, detection, mergers/separations) and spot matching.

Cy-dye automated analysis:

- Cross-stain analysis, Cy3:Cy5 groups Cy2 as internal standard, auto-selected reference gel, spots detected-default parameters, Progenesis background subtraction, warp and matched to reference combined warping and matching, unmatched spots added to reference and normalized to total spot volume.

PG 240 using SameSpots Analyses:

- Images warped using TT900 S2S v2006, needing 283-617 vector assignments. Warped images were processed using the automatic analysis wizard of PG240 and spot detection was corrected manually. The corrected spots were applied to all gels using SameSpots, followed by background subtraction and normalization by total spot volume.

DeCyder analysis:

- Gels were imported into the DeCyder differential in-gel analysis module. The spot number estimate used was 800. The spot maps for each gel were exported to the Biological Variation Analysis module where the gels were automatically matched and manually corrected.

Ludesi Analyses:

- Images uploaded to Ludesi 2D Analysis Center using the web-based software Ludesi 2D Interpreter. Proprietary image analysis software Ludesi 2D Analyzer used for spot detection, segmentation and matching. Correctness comparisons were made with Ludesi 2D Analyzer's built-in evaluation features and the results were visualized in Ludesi 2D Interpreter.

Correctness Comparison Criteria:

•Number of correct protein regulations:

- For each of the projects, ANOVA was performed
- The regulated proteins correctness was estimated by evaluating the detection, segmentation and matching correctness of the 50 proteins having the lowest p-values.
- The number of correct protein regulations was estimated as the correctness of the regulated proteins multiplied by the number of protein regulations presented by the different softwares at p=0.001.

•Spot detection correctness:

- 50 randomly selected spots in randomly selected gel
- Visually judged as satisfactory or not based on criteria:
 - False positive (noise, no spot)
 - Spot fragmentation (single marked as multiple, multiple as single)

•Spot matching correctness:

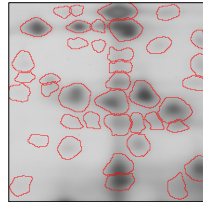
- For each of the projects, spots found in greater than 25% of gel images were extracted.
- 50 randomly selected spots per project
- Visually judged as satisfactory or not based on criteria:
 - Mismatch (incorrect match in ≥1 gels)
 - Missing match (absence of correct match in ≥1 gels)

•Spot segmentation correctness:

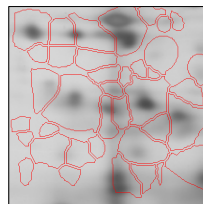
- 50 randomly selected spots in randomly selected gel
- Visually judged as satisfactory or not based on criteria:
 - Border enclosing whole spot
 - Border not enclosing parts of other spots

Spot Detection and Segmentation examples

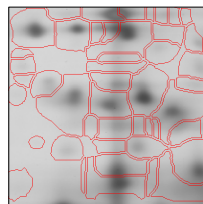
Silver-stained Serum Gels



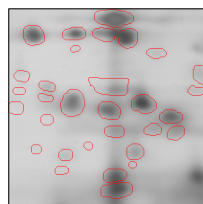
Ludesi



PG 240 using SameSpots

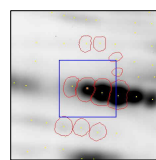


Progenesis auto

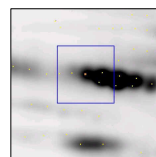


Progenesis manual

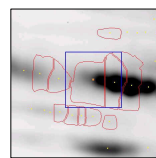
Cy-Labeled Serum Gels



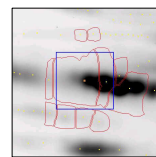
Ludesi



DeCyder

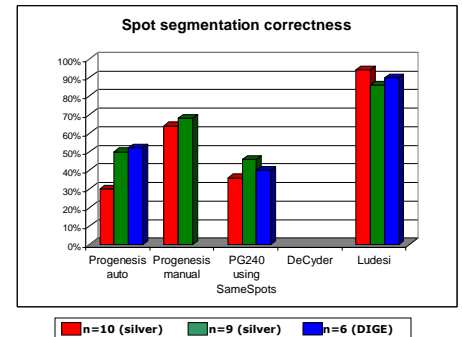
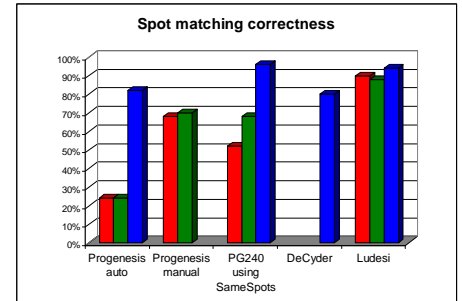
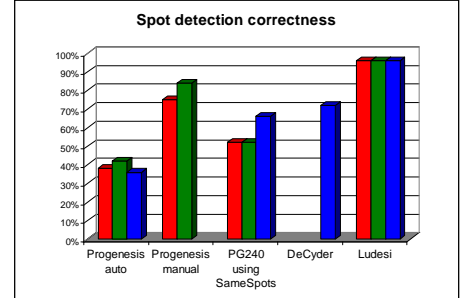
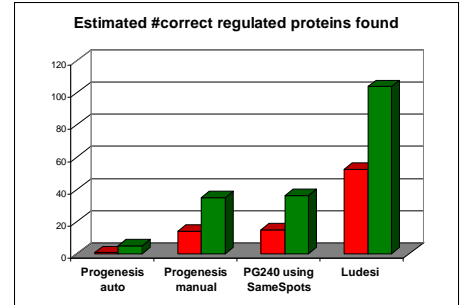


PG240 using SameSpots



Progenesis auto

Software Comparison Results



Legend: n=10 (silver), n=9 (silver), n=6 (DIGE)

Conclusions

- Ludesi provided the highest overall correctness for the serum gels analyzed, based on spot detection matching and segmentation. This will improve the ability of the 2D gel platform to track protein changes.

- Manual editing of Progenesis analyses improved the quality of analysis and is necessary when analyzing complex 2D gels.

- Complex serum gels with different concentrations and spot constellations make a Progenesis SameSpots alignment difficult.