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Quantitative comparison and evaluation of two commercially available, two-dimensional electrophoresis image analysis software packages, Z3 and Melanie

While a variety of software packages are available for analyzing two-dimensional electrophoresis (2-DE) gel images, no comparisons between these packages have been published, making it difficult for end users to determine which package would best meet their needs. The goal here was to develop a set of tests to quantitatively evaluate and then compare two software packages, Melanie 3.0 and Z3, in three of the fundamental steps involved in 2-DE image analysis: (i) spot detection, (ii) gel matching, and (iii) spot quantitation. To test spot detection capability, automatically detected protein spots were compared to manually counted, "real" protein spots. Spot matching efficiency was determined by comparing distorted (both geometrically and nongeometrically) gel images with undistorted original images, and quantitation tests were performed on artificial gels with spots of varying Gaussian volumes. In spot detection tests, Z3 performed better than Melanie 3.0 and required minimal user intervention to detect approximately 89% of the actual protein spots and relatively few extraneous spots. Results from gel matching tests depended on the type of image distortion used. For geometric distortions, Z3 performed better than Melanie 3.0, matching 99% of the spots, even for extreme distortions. For nongeometrical distortions, both Z3 and Melanie 3.0 required user intervention and performed comparably, matching 95% of the spots. In spot quantitation tests, both Z3 and Melanie 3.0 predicted spot volumes relatively well for spot ratios less than 1:6. For higher ratios, Melanie 3.0 did much better. In summary, results suggest Z3 requires less user intervention than Melanie 3.0, thus simplifying differential comparison of 2-DE gel images. Melanie 3.0, however, offers many more optional tools for image editing, spot detection, data reporting and statistical analysis than Z3. All image files used for these tests and updated information on the software are available on the internet (<http://www.umbc.edu/proteome>), allowing similar testing of other 2-DE image analysis software packages.

Keywords: Differential comparison / Proteome / Software package / Two-dimensional electrophoresis
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1 Introduction

Currently, 2-D gel electrophoresis is the technology most widely used [1, 2] to separate complex protein mixtures for subsequent differential comparison and proteomic analysis. To facilitate rapid and accurate differential comparisons, a number of specialized image analysis software packages have been developed. These packages have evolved from early versions which ran on DEC PDP 11 mini-computers (e.g., Elsie, LIPS, Gellab and Tycho) to Unix based packages (e.g., Gellab II, Elsie 4, Melanie 3.0, Quest) [3], to the latest generation of Unix, PC and Mac based pro-

grams shown in Table 1. The various software packages listed have a broad range of options, capability and price, making it somewhat difficult to choose the software best suited for ones needs. While a number of publications describe technical details related to individual packages [4–12] no work has been done to quantitatively compare these different packages using a common set of tests.

Our goal here was to develop a set of tests which could be used to quantitatively evaluate 2-DE image analysis software packages in the three fundamental steps involved in differential comparison: (i) spot detection, (ii) gel-to-gel matching and (iii) spot quantitation. In the first step, spot detection, the software package searches the gel image to determine whether a particular feature is, or is not, a protein spot. In this study, spot detection capability was determined by comparing automatically detected protein spots with manually identified "real" protein spots. The

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Table 1. Commercial software packages currently available for 2-DE gel image analysis^{c)}

# ^{a)}	Software	Company	Year of arrival	Cost ^{e)}	Comments	Platforms	Images supported
1	Delta 2D™	DECODON GmbH http://www.decodon.com	2000	US \$ 4185 ^{d)} (Euro 3750)	Save-disabled evaluation version available	PC [Windows 98, ME, 2000, NT], Linux, Sun Solaris, Mac OS X	TIFF (8, 12 and 16 bit), JPEG, BMP, GIF, PNG.
2	GELLAB II+™	Scanalytics http://www.scanalytics.com/	1999	US \$ 1995	Trial version available	PC [Windows 95, NT]	TIFF (8 bit)
3	Melanie™	Geneva Bioinformatics S.A. http://www.genebio.com	1991	US \$ 6500	30 day fully functional trial version available	PC [Windows 95, 98, 2000, NT]	TIFF (8, 16 bit), GIF, Bio-Rad Scan
4	PD Quest™	Bio-Rad Laboratories Inc. http://www.biorad.com	1998	US \$ 12500	30 day fully functional trial version available	PC [Windows 95, 98, NT], Macintosh Power PC	TIFF (8, 16 bit)
5 ^{b)}	Phoretix 2D Advanced™	Nonlinear Dynamics Ltd. http://www.nonlinear.com http://www.phoretix.com	1991	N/A ^{f)}	Trial version available through sales agent	PC [Windows 95, 98, 2000, NT]	TIFF (8, 12 and 16 bit)
5.1	AlphaMatch 2D™	Alpha Innotech Corporation http://alphainnotech.com	1999	US \$ 6788	Trial version available through sales agent	PC [Windows 95, 98, 2000, Me, NT]	TIFF (8, 12 and 16 bit)
5.2	Image Master 2D Elite™	Amersham Pharmacia Biotech http://www.apbiotech.com	2001	US \$ 7740	Trial version available through sales agent	PC [Windows 95, 98, 2000, Me, NT]	TIFF (8, 12 and 16 bit)
5.3	Investigator HT Analyzer™	Genomic Solutions Inc. http://www.genomicsolutions.com	2000	US \$ 8700	Trial version available through sales agent	PC [Windows 98, 2000, NT]	TIFF (8, 12 and 16 bit)
6	Progenesis™	Nonlinear Dynamics Ltd. http://www.nonlinear.com http://www.phoretix.com	2001	N/A ^{f)}	Special hardware and software requirements	PC [Windows 2000]	TIFF (8, 12 and 16 bit)
7	Z3™	Compugen http://www.2dgels.com	2000	US \$ 9000	21 day fully functional trial version available	PC [Windows 98, 2000, NT]	TIFF (8, 12 and 16 bit), JPEG, BMP, GIF, PNG, GEL, FLT

a) The software packages have been arranged in alphabetical order of their brand name.

b) The software packages listed under 5 are essentially the same as “Phoretix 2D Advanced™”, marketed under different brand names. Please contact individual companies to know about any differences, there may be.

c) The software packages listed in the table are only comprehensive-off-the-shelf-commercial-software-packages-available-for-2Dgel-image-analysis. The information listed in the table has been obtained from various sources, including internet, literature and sales agents. Misinformation, if any, is purely unintentional.

d) Calculated as per the conversion rate on 06–25–01.

e) Cost listed is the academic price of the software packages.

f) Not available

ability of software to detect as many real protein spots and as few extraneous spots as possible, with minimal user intervention, was used as the criteria for evaluation. In the second step, gel-to-gel matching, a differential comparison is carried out by matching spots (or features) that may be in slightly different locations (due to reproducibility problems) on the two gels. To evaluate capability in gel-to-gel matching, we compare artificially distorted gel images with the undistorted image of the same gel. The fraction of unmatched spots in the undistorted gel is then used as the criteria for evaluation. Others have used a similar approach with geometrically distorted

(i.e., by stretching and/or rotating) gels [13]. In actuality, however, many imperfections are nongeometrical, and thus testing in this study was carried out on gels with both geometrical and nongeometrical distortions, providing what we believe is a more accurate assessment of gel matching capability. Once gel-to-gel matching has been carried out, the software package is used to identify proteins expressed at different levels by quantifying the spot volume (spot-area-in-pixels × pixel-gray-level). To evaluate spot quantitation efficiency, we have generated a series of artificial gel images with “ideal” Gaussian-shaped spots of known volume. All of the images used in the tests

described here are available on the Internet (<http://www.umbc.edu/proteome>) and thus may be used to test any 2-DE gel image analysis software package. (Note, the computational speed for 2-DE image analysis was not one of the factors taken into consideration for evaluating the software packages in this study).

For this study, we have used the tests described above to compare two commercially available 2-DE image analysis software packages, Melanie 3.0 and Z3, which differ significantly [13]. For example, in Melanie 3.0, spot detection is the basic procedure for gel alignment and subsequent image analysis steps whereas Z3 uses region based registration of the raw images as their fundamental step. Melanie 3.0 is one of the most widely used software packages, as it was one of the first available (1991), and has undergone significant development over the past decade. In contrast, Z3 entered the market in 2000, employs automatic gel warping (or aligning) and utilizes a dual color display to aid in visualization of differences in protein expression [14].

2 Materials and methods

2.1 Software and gels

2-DE image analysis software packages, Melanie 3.0 version 3.03 (Geneva Bioinformatics (GeneBio) S.A., Geneva, Switzerland), and Z3 Versions 1.51 and 2.0 (Compu-gen Ltd, Tel Aviv, Israel), were used for studies in this paper. While Z3 1.51 was used in spot detection and gel matching studies, Z3 2.0 was used in spot quantitation studies. Two standard gels were used in spot detection and gel matching studies. Gel (a) is one of the many gels used by the Z3 manufacturers, in their studies in [13]. Gel (a) was downloaded from <http://www.2dgels.com/benchmark/> and cropped for use. Gel (b) is one of the gels sup-

plied for reference with the Melanie 3.0 package by their manufacturers. Gel (b) was obtained by exporting one of the *Escherichia coli* gels, "ECOLI.mel" to TIFF format and converting it to grayscale. Both gels, (a) and (b) are 8-bit, grayscale, TIFF images with image sizes of 518 and 1104 kB, respectively. The images can be downloaded from <http://www.umbc.edu/proteome>.

2.2 Spot detection analysis

The total number of real protein spots was counted manually. In order to do this, the contrast and brightness of the image was adjusted to help in better visualization of the protein spots. The images were then enlarged 5–10 times and the spots were manually circled for counting. For detailed methods and guidelines used for counting the protein spots manually, please refer to <http://www.umbc.edu/proteome>. Once the spots were identified manually, the spots were detected using default and adjusted settings (see Fig. 2 caption for details of settings) in Z3 and Melanie 3.0. For each of the settings, the software spot detection was compared to manual spot detection on a spot-by-spot basis to identify the spots missed (or not detected) by the software. The total number of extraneous spots was then calculated algebraically using the following formula: "extraneous spot count = software spot count – manual spot count + total missed spots". The total number of spots in each category was normalized, with the total number of real protein spots as 100% for plotting the data.

2.3 Gel matching

The gels were distorted using Adobe PhotoDeluxe Business Edition (PBE) Version 1.0 (Adobe Systems Inc., USA) and Quantity One (Q1) Version 4.2.2 (Bio-Rad Labora-

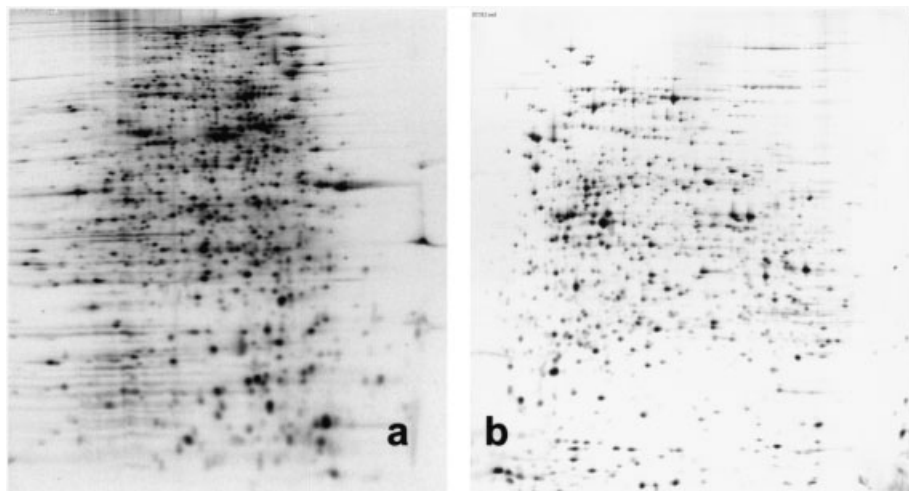


Figure 1. Two standard gels used in spot detection and gel matching studies. (a) Gel image downloaded from <http://www.2dgels.com/benchmark/> (b) Gel image from Melanie 3.0 package. The above gels can be downloaded from <http://www.umbc.edu/proteome>.

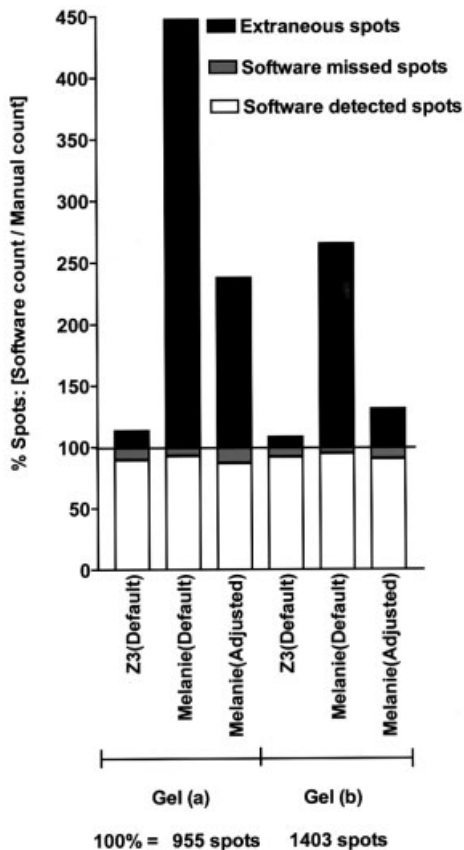


Figure 2. Spot detection analysis of gel (a) and gel (b) using Melanie 3.0 and Z3 1.5. (i) Total spots detected by the software, expressed as percentage of the manual count, plotted for different detection settings of the two software packages. Automatic default settings were used for Z3. Melanie 3.0 default settings: Number of smooths 2, Laplacian threshold 3, partials threshold 1, saturation 90, peak-ness increase 100, minimum perimeter 10; Melanie 3.0 adjusted settings [Gel (a)]: Number of smooths 3, Laplacian threshold 3, partials threshold 1, saturation 90, peak-ness increase 100, minimum perimeter 13; Melanie 3.0 adjusted settings [Gel (b)]: Number of smooths 4, Laplacian threshold 3, partials threshold 1, saturation 90, peak-ness increase 100, minimum perimeter 14.

tories (Hercules, CA, USA), software packages. For “height decrease distortions”, the total height in the SDS dimension was decreased using “photo size” under advanced-size options in PBE. The center pull distortions were created using “shear” under advanced-special effects in PBE. The % distortion for each gel was calculated using Q1 as described in Fig 3 caption. The distorted images can be downloaded from <http://www.umbc.edu/proteome>. The original gel image was matched to each of the distorted images using both Melanie 3.0 and Z3. For Melanie 3.0 matching studies, the spot detection settings used can be seen in the captions to Figs. 4 and 5.

In both gels (a) and (b), 20 well-distributed spots were randomly selected and used as landmarks in matching. Multiple pairing (one-to-many and many-to-one spot matching) was allowed (Note, allowing only one-to-one spot pairing did not affect the results obtained in this paper). After matching, the spot pairs were highlighted and the unmatched spots in the original image were manually counted. In Z3 matching studies, most of the distorted gels could be automatically warped (aligned) to the undistorted image. Some of the images with high distortions required one anchor initially for the gels to be registered automatically. For Z3 (adjusted), anchors were manually added after automatic registration to improve gel matching. The total number of unmatched spots was estimated by counting those spots, which were present only in the reference-undistorted gel after matching. In matching studies with “height-decreased gels” spots unmatched in 100% region of the gel was counted. Whereas, for center pull distorted images only the spots unmatched in the top 90% of the gel in the SDS dimension was counted. This was done to avoid those spots, which were unmatched due to the absence of the corresponding spots in the distorted images, especially at very high distortions. The percentage of spots unmatched in the undistorted image was used for plotting the data.

2.4 Spot quantitation studies

Artificial gels were generated using MATLAB Version 6.01 programming language, The MathWorks Inc. (Massachusetts, USA). Each gel contained a total number of 13 spots, with one spot in the center and 12 spots geometrically distributed around the image. The spots were modeled using the two-dimensional Gaussian distribution function. The Gaussian volume (spot-area-in-pixels \times pixel-gray-value) of the center-spot was changed across different gels while the others were held constant. The peak height of the Gaussian, corresponding to the highest gray value, was the same for all the spots. A total of 11 gels (a) through (k) were generated with the volume ratio of their center spot, as compared to the center spot in gel (a) being 2, 4, 6, 10, 14, 18, 22, 26, 30, and 40, respectively. The program was designed to generate 300 dpi, 8-bit, TIFF images and also, to calculate the spot area and the Gaussian spot volume. The program and images are available for downloading from <http://www.umbc.edu/proteome>. In Melanie 3.0, four of the 12 nonchanging spots were used as landmarks for gel matching. In Z3, gels were analyzed in the “multiple gel analysis mode”. Spot detection parameters were adjusted to detect all the spots. The set of 12 nonchanging spots were used for calibration in Z3. The differential expression values were compared with the known spot ratios.

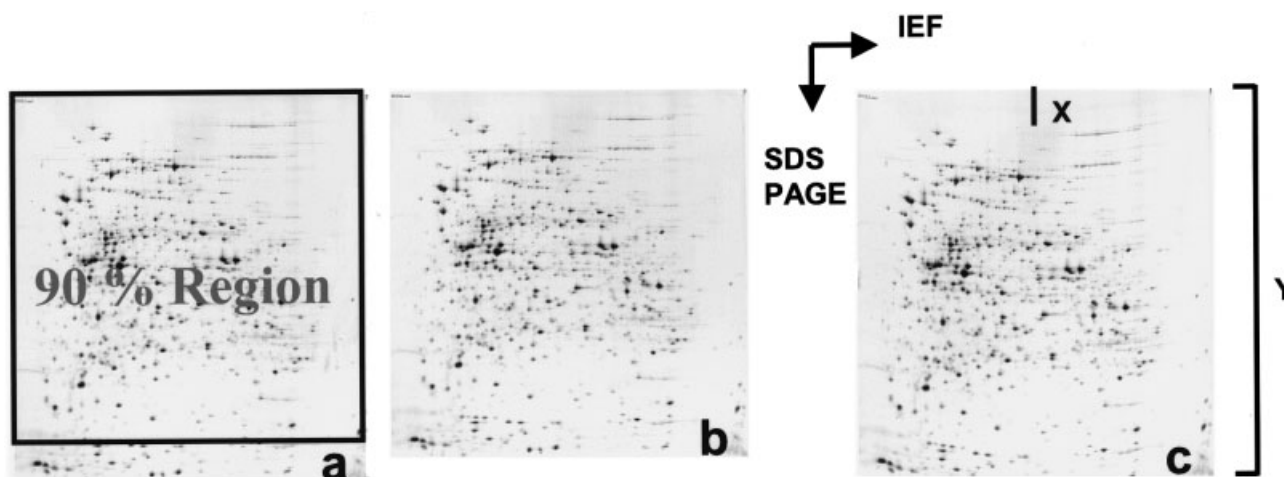


Figure 3. Example of “height decrease” and “center pull” distortions. (a) Original undistorted gel, (b) “height decrease” distorted gel, (c) “center pull” distorted gel. Maximum distortion is at the center of the image, marked “X”. Percentage “center pull” distortion = $X/Y \times 100$. Note: In matching studies between the original and “center pull” distorted image, spots unmatched in the “90% region” of the original undistorted image were taken into account.

3 Results and discussion

3.1 Spot detection

In the analysis of 2-DE gel images, spot detection is the first, and may be the most fundamental step involved. This is because optimal detection of protein spots is necessary for efficient execution of subsequent steps (*i.e.*, spot matching and quantitation). Ideally, 2-DE image analysis software packages should be able to identify all protein spots in any given gel. However, in reality some protein spots can be missed, while extraneous nonprotein spots may be detected. In both cases, user intervention is required for optimal spot detection. Thus, in our comparison study, the criteria for evaluating spot detection efficiency of the two software packages is their ability to detect protein spots while avoiding extraneous spots, with minimal user intervention.

As described in Section 2, a quantitative test was devised to determine how well image analysis software packages detect protein spots. This test was then applied to both Melanie 3.0 and Z3. As shown in Fig. 2, Z3 detected more than 89% of the protein spots and a maximum of only 14% false spots, in both gels (a) and (b), using “automatic” default spot detection settings. Most of the missed spots were in clustered or streaked regions, where multiple spots were often detected as a single spot. Although Z3 gave specific reasons for missing protein spots (Z3 highlighted reasons, such as, “weak, touches background, segment inside other, bad symmetry *etc.*,” when the cursor was pointed over undetected protein spots), only some of them had corresponding adjustable parameters to improve spot detection. Be-

tween the two standard gels used, Z3 detected a slightly higher percentage of protein spots and fewer extraneous spots in gel (b). Also shown in Fig. 2 are results from Melanie 3.0, which detected more than 93% of the protein spots in both the gels with default settings. However, Melanie 3.0 also detected many extraneous spots, 350% in gel (a) and 165% in gel (b). A significant number of missed spots were in clustered regions. But, unlike Z3, Melanie 3.0 provides a specific adjustable parameter to tackle the problem of spot detection in such streaked regions. Most of the extraneous spots detected were minute specks (perhaps due to staining artifacts). In order to reduce the number of extraneous spots and to further optimize the spot detection, two of the spot detection parameters were adjusted. The “number of smooths” on the image before spot detection and the “minimum perimeter” required for a feature to be detected as a spot, was increased. This reduced the percentage of extraneous spots detected to 138% in gel (a) and 31% in gel (b), as shown in Fig. 2 for Melanie 3.0 (adjusted). This was accompanied by a maximum loss of only 6% in the protein spots detected. This shows that in Melanie 3.0, by manually adjusting various parameters, spot detection can be significantly improved.

In summary, Z3 did a comparably better job than Melanie 3.0 in spot detection. Using default settings, Z3 detected most of the real protein spots and relatively few false spots, reducing user intervention required for optimal spot detection. While Melanie 3.0 offers more adjustable spot detection parameters than Z3, using these parameters requires a considerable amount of user intervention, due to the high fraction of extraneous spots. Note that both Z3 and Melanie 3.0 did better with gel (b), which

is cleaner than gel (a), implying image quality is important for better spot detection, and image clean up may significantly improve gel analysis. Even with optimized spot detection, irrespective of the software package used, some amount of user intervention in the form of manual addition or deletion of spots may be required.

3.2 Gel matching

Obtaining high-quality 2-DE gels remains an art, as differences can arise from a number of factors (gel running conditions, temperature effects, uneven focusing, polymerization problems, etc.). As a result, it can be quite challenging to achieve a high degree of reproducibility between gels. To compensate for this, 2-DE image analysis software should be able to account for variations during the gel-to-gel matching process. To study gel-to-gel matching efficiency, we compared our original, undistorted images with distorted images of the same gels (Fig. 3). In the first study, undistorted images were compared with images modified by decreasing the entire height in the SDS dimension (Fig. 3b). This type of geometric distortion is similar to that which might occur due to differences in gel pouring, which would alter the height of the slab gel. In the second study, the same undistorted images were compared with images modified by pulling the center of the gels in the SDS dimension (Fig. 3c). This type of nongeometric distortion is similar to that which might occur in gel runs due to temperature effects. While the high degree of distortion in some of the gels used in this study is not common in reality, evaluation under these conditions sheds light on the extent to which software can account for distortions of varying degree. After gel-matching of undistorted and distorted images, performance was evaluated by determining the fraction of spots unmatched in the original undistorted image [13].

Figure 4 shows matching results for tests performed on gels with decreased height. Melanie 3.0 shows a significant improvement in gel matching (*i.e.*, lower percentage of unmatched spots) when optimized spot detection settings (*i.e.*, “settings II”) are used. Recall that in the spot detection analysis described above, adjustment of Melanie 3.0 parameters led to a significant decrease in the number of extraneous spots detected. Thus, many of the unmatched spots in Fig. 4 may be due to extraneous spots detected by Melanie 3.0, leading one to conclude that optimal spot detection, with minimal false spots, may be required for good gel matching. In contrast, Z3 did an outstanding job in automatically matching (without any user intervention or parameter adjustment) as many as 99% of spots, even at the maximum distortion of 5%. Thus, we conclude for the geometric distortions Z3 did better job than Melanie 3.0 at gel-to-gel matching.

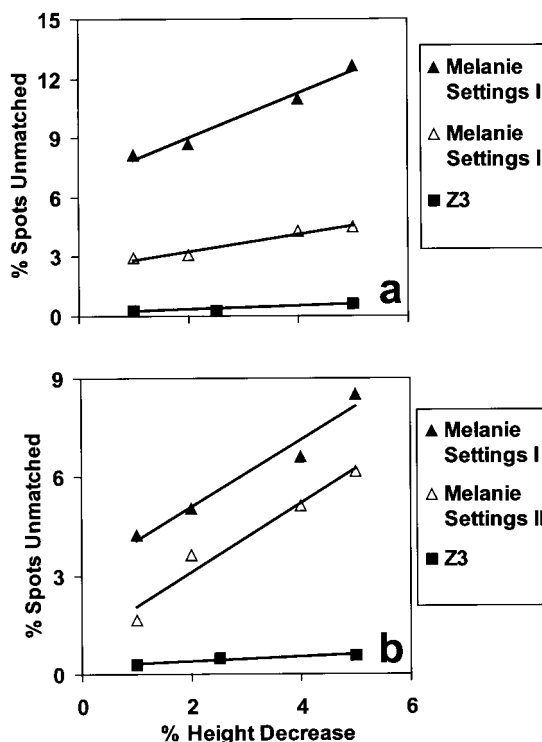


Figure 4. Matching studies for Gel (a) [top] and Gel (b) [bottom] for comparison between undistorted regular image and distorted (height-decreased in SDS-PAGE dimension) image. Percentage spots unmatched in the original image, after comparison, is plotted against % distortion. (▲) Melanie 3.0 spot detection settings I: Number of smooths 3, Laplacian threshold 3, partials threshold 2, saturation 90, peakedness increase 100, minimum perimeter 12; (△) Melanie 3.0 spot detection settings II: Number of smooths 4, Laplacian threshold 3, partials threshold 3, saturation 90, peakedness increase 100, minimum perimeter 12; (■) Z3 matching without user intervention.

Figure 5 shows matching results for tests performed on gels altered by “center pull.” As in the previous case, Melanie 3.0 shows a significant improvement in gel matching performance when optimized spot detection settings (*i.e.*, “settings II”) are used. In addition, tests on gel (b), which has a lower number of extraneous spots than gel (a), show a lower percentage of unmatched spots. Taken together, these results again imply that many of the unmatched spots in the Melanie 3.0 gel matching tests may be due to the large number of extraneous spots detected by Melanie 3.0. Note that the slope of the linear fits for Melanie 3.0 tests is small, implying Melanie 3.0 has the ability to accommodate a high degree of center pull distortion. Hence, if spot matching (*i.e.*, the step preceding gel-matching) is improved, and the number of false spots detected is minimized, Melanie 3.0 has the potential to match gels very efficiently. In the case of Z3, with

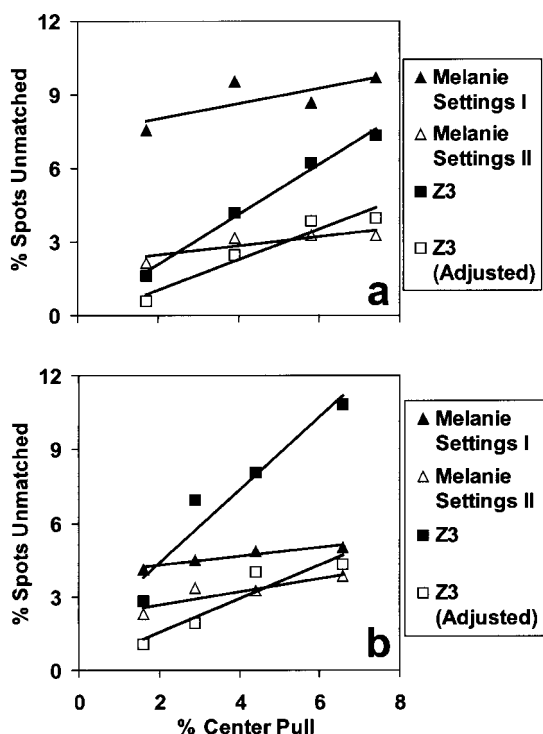


Figure 5. Matching studies for Gel (a) [top] and Gel (b) [bottom] for comparison between undistorted regular image and distorted (center pull) image. Percentage spots unmatched in 90% of the original image, after comparison, is plotted against % distortion. (\blacktriangle) Melanie 3.0 spot detection settings I: Number of smooths 3, Laplacian threshold 3, partials threshold 2, saturation 90, peakedness increase 100, minimum perimeter 12; (\triangle) Melanie 3.0 spot detection settings II: Number of smooths 4, Laplacian threshold 3, partials threshold 3, saturation 90, peakedness increase 100, minimum perimeter 12; (\blacksquare) Z3 matching without (or minimal) user intervention; (\square) Z3 matching with user intervention.

default parameters, the percentage of unmatched spots shows a steep increase with increasing degree of center pull. This implies lower resilience than Melanie 3.0 to this type of distortion. However, when Z3 “registration anchors” (similar to landmarks in Melanie 3.0) are added manually, there is a significant reduction in both the percentage of unmatched spots and the slope of the linear fits. In this case, less than 3% of the spots were unmatched for up to 5% distortions.

In general, gel-to-gel matching involves two steps: gel alignment or “warping” followed by spot matching. Z3 and Melanie 3.0 differ in the first of these steps – gel alignment. Z3 performs automatic, whole-gel “warping”, using similar rectangular regions between the two gels to align them [13]. With Z3, spot detection is not required for proper gel alignment. As a result, most of the distorted

gels could be “automatically” warped (aligned) with the original gel, and thus Z3 required minimal user intervention and showed a high degree of success. Note that in all Z3 tests, most of the unmatched spots were around the periphery of the gel image, implying the whole-gel warping technique used by Z3 works better in regions where there is a high concentration of spots. This was confirmed by the fact that in a central region of the gel (*i.e.*, half the dimensions of the original image) less than 5% of the total number of unmatched spots was present for any given distortion (data not shown). In contrast to Z3, spot detection is required with Melanie 3.0 for proper gel alignment. In addition, Melanie 3.0 alignment relies on user identification of common spot pairs (landmarks) between gels [15]. Hence, Melanie 3.0’s ability to properly align gels and match spots depends on the intelligent choice of well-distributed landmarks by the user. Together, these factors make gel alignment in Melanie 3.0 somewhat exhaustive, requiring considerable user intervention.

In summary, Z3 did a better job of gel matching for geometric or height decreased distortions. However, for gel matching of nongeometric or center pull distorted images, both software packages did comparably well after user intervention. Note however that gel alignment in general is comparably easier, requiring significantly less user intervention in Z3 than in Melanie 3.0.

3.3 Quantitation studies

Spot quantitation is perhaps the most important step in 2-DE image analysis, as it can provide valuable information on the differential expression of proteins between samples. In general, quantitative evaluation is based on differences in the relative intensity or darkness of protein spots, which can be related to differences in protein expression between given samples. In reality, many factors can interfere with quantitative evaluation, including: sample loading errors, irreproducibility of staining, protein losses at different stages of gel running, and differences in scanning properties. Researchers [16] have studied the effect of some of these factors on gel quantitation. For our quantitative comparison study, we have excluded all the above errors by using artificial gels (Fig. 6) in which the Gaussian volume (spot-area-in-pixels \times gray-level) of one spot is changed across gels, while all other spots are held constant. While a previous study used a similar approach [17], only one artificial gel was used. Our approach, using multiple artificial gels, allows study of the efficiency of software in predicting expression differences without interference from other variables. It should be noted that we have used only ideal-Gaussian-distributed spots in our studies with artificial gels. In reality, protein spots may not be Gaussian in nature. However, this simplified

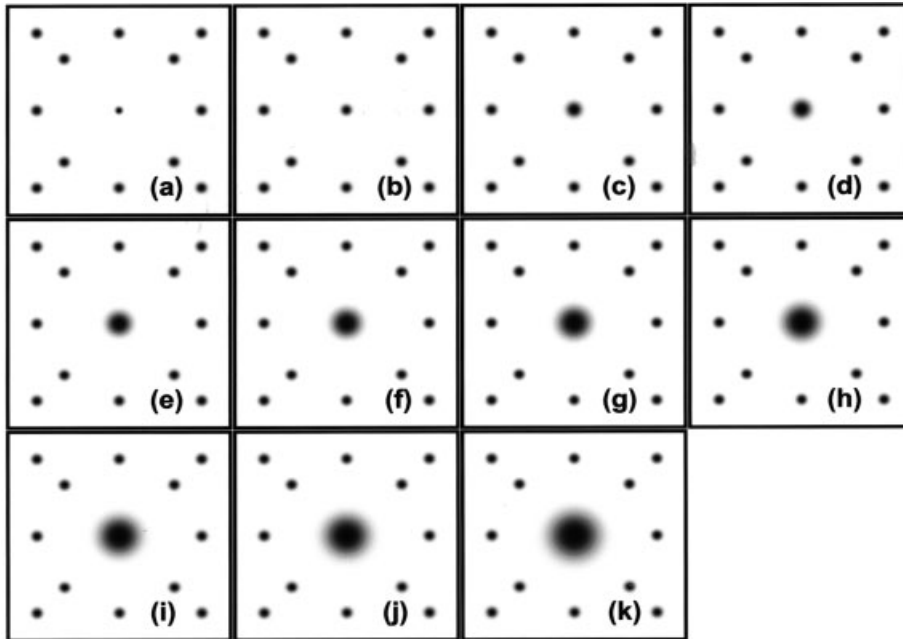


Figure 6. Artificial gels used for quantitation analysis. The images (300 dpi, 8-bit, .tiff) were generated using MATLAB 6.01. The Gaussian volume of the spot in the center was varied across gels. The volume ratio of the center spot in gels (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), as compared to the center spot in gel (a) was 2, 4, 6, 10, 14, 18, 22, 26, 30, 40, respectively. Images can be downloaded from <http://www.umbc.edu/teome>.

method using completely defined spots allows a user to assess basic quantitation limitations, if any, in the software packages.

Figure 7 shows the results from our quantitative comparison study. Melanie 3.0 did a relatively good job of estimating spot ratios over the entire range of values tested, with a slope of 0.86. In contrast, Z3 predicted spot ratios fairly well, until spot ratios that exceeded 6. For spot ratios between 10–40, Z3 estimated differential expression ratios that were one-fourth the actual value. Also note that the values plotted for Z3 were obtained after calibration of the 12 nonchanging spots. The predictions were even worse without calibration. In summary, Melanie 3.0 did a relatively good job at predicting spot ratios over the entire range tested, while Z3 significantly under predicted spot ratios when values were greater than 6.

4 Concluding remarks

Melanie 3.0 and Z3 performed differently in the three categories tested. While Z3 was more efficient in spot detection, detecting most real protein spots and few false spots, requiring minimal user intervention, Melanie 3.0 was able to predict differential protein expression between gels much better than Z3. The performance of the two packages in gel matching was dependent on the type of distortions studied.

Melanie 3.0 is more than a decade old, and many significant features have been added during this time. Melanie 3.0 has many more optional tools than Z3 for image

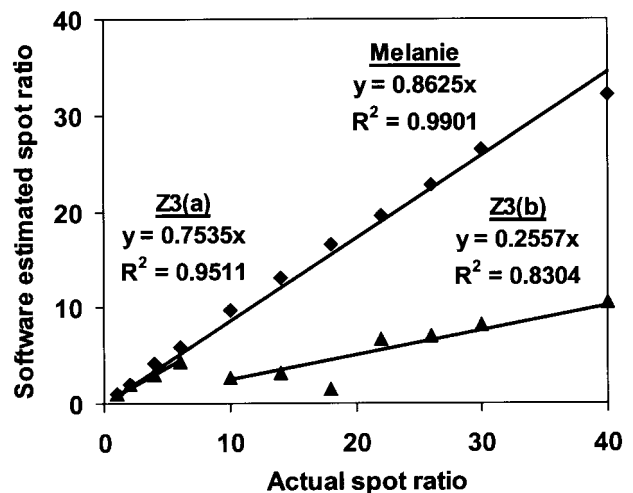


Figure 7. Quantitation analysis: comparison of spot ratio estimated by the software packages (◆ Melanie, ▲ Z3) to that computed using the MATLAB Gaussian volumes for the center spot in the artificial gel images. The data for Z3 analysis is fitted with two trendlines, Z3(a) and Z3(b) for data between spot ratios 1–6 and 10–40 respectively.

editing and data reporting. Melanie 3.0 also offers a variety of parameters for optimizing spot detection, however, this requires a significant amount of user intervention. The statistical tools available in Melanie 3.0 are very useful for robust data analysis. Melanie 3.0 is also connected with the well-annotated SWISS-PROT protein sequence database, which can be used to directly query for protein identifications. Z3 is relatively new software, which has been in the market only for two years. Its automatic matching

algorithm is much faster and easier to use than landmarks based gel alignment in Melanie 3.0. It offers great promise in making image analysis a less time consuming and laborious task.

In summary, it can be said that the two software packages tested here have individual strengths and weaknesses. Hence, common platforms where multiple software packages can be integrated will be of great significance, offering users the option of using different packages for different steps in the image analysis of 2-DE gels, based on the pros and cons of each software package. However, cost may be a constraint. While only two packages have been evaluated in this study, the methods outlined in this paper and images used for testing are available on the internet (<http://www.umbc.edu/proteome>) and may be used by others to evaluate different commercially available software packages, based on specific needs and also for the research done.

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