Comparative evaluation of two two-dimensional gel electrophoresis image analysis software applications using synovial fluids from patients with joint disease

PANKAJ S. ARORA1, HIROSHI YAMAGIWA1,2, ALOK SRIVASTAVA1, MARK E. BOLANDER1, and GOBINDA SARKAR1

1Department of Orthopedic Research, 3-93 Medical Science Building, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
2Division of Orthopedic Surgery, Department of Regenerative and Transplant Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Abstract The proteomic composition of synovial fluid (SF) may hold clues to understanding the molecular basis of arthritis. However, the highly viscous nature and proteomic complexity of SF present a challenge when analyzing results obtained by two-dimensional gel electrophoresis (2D-GE). Several software applications are available for analyzing 2D-GE images. Despite inherent strengths and weaknesses, no comparison between these applications has been reported using SF or any human fluid specimens. We evaluated two common software packages — PDQuest and Progenesis Workstation — for spot detection and Progenesis for spot detection, matching, and quantitation of 2D-GE images of SF from four patients with arthritic disease. Initially, whole 2D-gel images were analyzed for spot detection, which suggested that PDQuest is more consistent than Progenesis; however, PDQuest appeared to require more user intervention than Progenesis. Subsequently, two small areas (spots well resolved and spots not well resolved) were selected from each gel image, which were analyzed by the software for spot detection, matching, volume, and resolution. These analyses suggest that both tools can quantify well-resolved spots relatively consistently when compared with manual spot detection (the “gold standard”). The “3D viewer” option offered by both tools enables correct spot identification and matching. The strengths and weaknesses of these computer tools can provide guidance in the choice of a particular workstation for identifying biomarkers of arthritis.

Key words Two-dimensional gel electrophoresis (2D-GE) · PDQuest · Progenesis · Image analysis

Introduction

Two-dimensional gel electrophoresis (2D-GE) is a powerful and widely used research tool in proteomics.2–4 Investigators in this area of research are faced with the challenge of analyzing protein spots in gels produced by 2D-GE. Typically, a single 2D-GE gel may be comprised of hundreds to thousands of protein spots. The comparison of protein spots between gel images includes detection of protein spots, matching spots between images from different gels, and quantitation of spots between matched images. Accuracy in spot detection and matching determines the quality of results obtained. It is impractical to quantitate and analyze large numbers of spots manually; therefore, a number of sophisticated automated image analysis software tools have been developed commercially.6,7 Because different software applications employ different spot detection and matching methodologies, it is difficult to decide on a specific tool.

Our long-term goal is to identify biomarkers for joint diseases through proteomic analyses of synovial fluid (SF).2 However, the success of such an investigation depends considerably on the effectiveness of the available tools for analyzing biological fluid specimens from patients. Clearly, the most efficient and economically manageable tools must be employed to ensure success in a timely manner. Furthermore, to our knowledge, no comparative evaluation between software applications has been reported using human fluid samples.

Two recent reports described their results on addressing this issue and provide useful guidelines. Raman et al. compared two software applications (Z3 and Melanie 3.0) in three steps: (1) spot detection; (2) gel matching; and (3) spot quantitation using 2D images that were available online.6 They concluded that each of these software applications has individual strengths and weaknesses. Nishihara and Champion evaluated three image analysis software applications (Z3, Progenesis, PDQuest) to check the sensitivity and linear dynamic range of 2D-GE.5 These investigators restricted their analysis to purified recombinant proteins. They concluded that Progenesis and PDQuest were more suitable than Z3 and that they produced more comparable...
results. Although these evaluations were based on a small number of proteins, they still provided valuable practical guidelines. However, none of these investigators analyzed complex mixtures of proteins such as can be expected from human body fluids.

In real-life situations, investigators are often confronted with a scenario in which the same amount of total protein from different protein sources (e.g., body fluids from different patients, differently treated cell cultures) are compared by 2D-GE to detect the changes of protein profiles. The position and quantity of protein spots are unknown in this type of analysis. This lack of information regarding the number of spots adds to the challenge of comparing the performance of different tools. We believe that comparative evaluation of software applications must be carried out with samples where both the complexity and concentration of individual proteins are unknown. Such an analysis should not only have implications in the choice and use of a particular software applications but can also help software developers improve their products.

Our objective was to evaluate two commonly used software applications using 2D-GE images of human synovial fluid from patients with joint disease. The software applications were compared for detection, matching, and quantitation performance. Our observations should have implications for other investigators analyzing SF for various reasons.

Materials and methods

Patients and sample preparation

Protein samples of human SF from four patients with joint disease (arthritis) were collected under the approval of an institutional review board, and informed consent was obtained prior to collection. Two patients (refers to image numbers 1 and 2 in Table 1) had anterior cruciate ligament (ACL) injury. The first patient was a man 27 years of age, and the second was a woman 21 years of age. Two other patients — one man 40 years of age and a woman 54 years of age (refers to image numbers 3 and 4) — had rheumatoid arthritis (RA).

Image acquisition

Separate 2D-GE on each of the four patient SF samples (100μg of human SF protein) were performed followed by staining with SYPRO Ruby fluorescent stain as previously described.12 After staining, each gel was scanned using PDQuest version 7.1.1. This version of PDQuest has support for scanning 2D images with compatible scanners. In our study, we scanned images at 100μm resolution using a Molecular Imager FX scanner (Bio-Rad Laboratories, Hercules, CA, USA). The scanned images were saved in the format prompted by PDQuest. The images were then converted to tagged image file format (TIFF) files for use by Progenesis Workstation. This is a standard approach and file format allowing for the transfer of an image from PDQuest to another software application.

Spot detection by PDQuest

The built-in pepper (noise) filter was applied to the imported images in PDQuest with the following settings: Outlier, 5×5, Salt Filter Off. PDQuest was then appropriately calibrated; a large spot and a faint spot were chosen for spot detection, as displayed in Fig. 1. This resulted in PDQuest choosing the following parameters: 16.68 for sensitivity, 5 for size scale, and 87 for minimum peak. After setting the parameters, PDQuest was used to determine the number of spots on each of the four gels.

Table 1. Number of spots detected in whole gel images by PDQuest and Progenesis

<table>
<thead>
<tr>
<th>Image no.</th>
<th>PDQuest</th>
<th>Progenesis</th>
</tr>
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<tbody>
<tr>
<td>1 (patient 1)</td>
<td>1080</td>
<td>884</td>
</tr>
<tr>
<td>2 (patient 2)</td>
<td>1040</td>
<td>1193</td>
</tr>
<tr>
<td>3 (patient 3)</td>
<td>1074</td>
<td>930</td>
</tr>
<tr>
<td>4 (patient 4)</td>
<td>1061</td>
<td>1250</td>
</tr>
</tbody>
</table>

Spot detection was carried out as described in the Methods section. Images 1–4 represent 2D-gel images obtained from analyzing the SF from patients 1–4, respectively.

Fig. 1. Representative image of two-dimensional gel electrophoresis of synovial fluid from patient 1. Areas A and B were selected as the “easy” and “difficult” areas, respectively, and were chosen for analysis based on their spot and clustering characteristics. The spots marked Large (red) and Faint (blue) were selected and used by the Spot Detection Parameter Wizard to calibrate PDQuest’s parameters.