The rapid progress of mass spectrometry (MS) development in proteomics allows now the identification of minute protein and/or peptide quantities. Correspondingly, much importance must be placed on how protein samples are handled from preparation through 2DE separation to preparation for MS analysis. Minor contamination by other proteins, e.g. due to imprecise spot picking, affects MS analysis. In addition, the probability of identifying a protein of interest is greatest when the greatest possible quantity of that protein is cut out. As a result, careful image evaluation is required based on high quality images.

2D gel electrophoresis has been established as a method for protein isolation since its first description in 1975 (1, 2, 3). As the various genome sequencing projects reach the final stages of completion, increasing amounts of data necessary for protein identification by MS analysis is becoming accessible. Considerable progress in the sensitivity and throughput of MS requires improvements to, and automation of, 2DE separation. If picking, digesting and spotting robots were still “exotic items” in lab automation a few years ago, today they are indispensable components of every modern proteomics lab. In the following aspects of spot picking and its affects on MS analysis are discussed with particular attention paid to image resolution and XY positioning accuracy.

2DE Proteomics Work Flow
After 2DE, the proteins in the gel must be stained. Fluorescent dyes such as SYPRO® Ruby (PerkinElmer Life Sciences) are used increasingly for this purpose. The sensitivity of SYPRO Ruby is similar to that of silver staining, but has a substantially greater dynamic range and is MS compatible (4). For visualization, SYPRO Ruby-stained gels are detected using imaging systems (e.g. ProXPRESS® Proteomics Imaging System, PerkinElmer Life Sciences) that can acquire the fluorescent image with high resolution, data depth and sensitivity.

Image analysis is then done using analysis software. However, the results of these conventional programs must be manually edited in a laborious, time-consuming manner. A new 2D image analysis

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Abbreviations:
MS: mass spectrometer, 2D: two-dimensional, 2DE: two-dimensional electrophoresis
package is now available (Progenesis, PerkinElmer Life Sciences) making the tedious editing superfluous and fully automating the image analysis process for the first time. With the corresponding computing power, this analysis now only takes hours instead of days or weeks. During image analysis, protein spots are selected, e.g. by differential measurements (control, sample) whose quantity (expression) has changed under controlled conditions.

The selected protein spots are then cut out of the gel using picking robots (e.g. ProPic™, PerkinElmer Life Sciences). After trypsin digestion, the peptides are eluted from the gel plugs (e.g. ProGest, PerkinElmer Life Sciences). Finally, the peptides are processed using preparation robots (e.g. ProMS, PerkinElmer Life Sciences) and then analyzed by MS.

Aspects of gel imaging

The quality of a 2D gel image, i.e., the sensitivity and dynamic range, is determined by the following factors: (1) sample handling, (2) staining method and protocol, and (3) performance of the imaging system. In order to quantify protein spots precisely using an analysis program, the highest possible spatial image resolution should be chosen so that as many measuring points as possible are obtained per spot and statistically significant results are thus obtained. It is advisable, however, to consider the resolution required relative to the file size. The image file of a large-format 2D gel with 16 bit depth and 100 µm spatial resolution is roughly 15 MB, and for 50 µm it is close to 60 MB. As a result, images of 2D gels are usually acquired using 100 µm resolution and 16 bit. This setting allows users to precisely quantify the protein spots and to differentiate between protein spots located close together, e.g. by detection of shoulders in spot intensity. Since the picking robot gets the XY coordinates of the protein spots from the 2D gel image, the resolution should concur with the XY positioning accuracy of the picking robot.

Aspects of spot picking

The spots to be excised can be determined either by direct marking in the gel (spot, click and cut) or via user-defined selection criteria in the analysis program. The protein spot locations are exported as a picking list to the picking robot. The user can then decide whether the spots in the geometric center or in the position of the largest quantity of protein should be cut out. Specifically, in the case of closely packed or overlapping spots, it is important to position the picking head precisely at the location selected. This requires the XY positioning accuracy of the picker to correspond to the spatial image resolution, with 100 µm spatial image resolution the positional accuracy should be also 100 µm to achieve consistently high quality sample preparation for MS. Positioning accuracy levels of more than 100 µm may lead to the cutting of protein spots that are contaminated by other proteins.

However, the gel must first be matched to the image from which the picking coordinates were determined. Because the gel matrix (and thus the spot positions) may change, for example, due to swelling, the current spot positions must be determined from the gel. For this purpose, either (1) a new image of the gel is acquired by the picking robot or (2) anchor points are used that are detected in the analysis image as well as by the picker.

(1) The imaging system in the picker should be able to detect not only visible gel stains, i.e. silver stain and Coomassie Brilliant Blue, but also fluorescent gel stains such as SYPRO Ruby. To ensure this, the picking robot must have the following components: CCD camera, UV and white light illumination for excitation, emission filters and a lightproof measuring chamber. For design and cost-related reasons, the imaging system in the picking robot cannot achieve the performance of an efficient, dedicated fluorescence imaging system. Rather, it is used for detecting protein spots that serve as anchor points. This has the advantage that actual spot positions are detected. In addition, for quality control, the result of the cutting can be directly monitored (Fig. 1).

(2) If anchor points are used, these must either be inserted in the gel or applied on the foils of plastic backed gels. Anchor points embedded in the gel can lie over spots and under certain circumstances do not represent all changes within the gel matrix. The foil of plastic backed gels keeps the gels in form and prevents them from changing its shape. Nevertheless, then only half of the gel surface is available for the stain to permeate. This can lead to a decrease in sensitivity.

Protein excision from plastic backed gels has a peculiarity: The picking head must be able to carry out a lateral movement in order to detach the gel plug from the foil. This movement should be as short as possible so as not destroy the gel or obscure the cutting of other proteins in the immediate vicinity. The length of this lateral movement depends on the positioning accuracy of the picking robot. For this reason as well, a high degree of positioning accuracy - 100 µm - is of decisive importance for MS analysis of proteins.

Closing remarks

Proteomic mass spectrometry requires a consistently high level of sample preparation. The results of sample preparation in the areas of gel imaging and spot picking can ultimately determine the outcome of the final mass spectrometric analysis.

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