
Preface

As commonly acknowledged, two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) is one of the most widespread techniques for the separation of complex protein extracts, above all in the research field, for the identification of candidate biomarkers of different biological effects (pathologies, drug effects, ripening effects, etc.). Notwithstanding its being quite time-consuming and laboratory intensive, it is still one of the most exploited techniques for the separation of protein extracts due to its low cost as compared to high-throughput high-resolution methods, and its versatility. SDS 2-D PAGE can also be considered nowadays as a phase of sample pre-treatment to enhance or facilitate the subsequent analysis based on mass spectrometry, and remove the masking effect of the most abundant proteins.

The achievement of the final 2-D map is based on a multistep experimental procedure involving: sample preparation and pretreatment, isoelectric focusing, interfacing of the first and second dimension, separation according to the molecular mass, and staining and destaining of the final 2-D map. Once the final maps are obtained, they have to undergo a final step usually named “differential analysis,” providing sets of candidate biomarkers by comparison of maps from different classes of samples (e.g., control vs. pathological, control vs. drug treated etc.). Actually, this final step is in turn based on a multistep complex procedure based on the exploitation of image analysis tools to provide information about the differences existing between the groups of investigated samples, i.e., the candidate biomarkers. In most cases the comparison of different 2-D maps is accomplished by dedicated software packages guiding the operator through a wizard-like procedure for noise removal (background, artifacts, etc.) and image warping, with the final aim of aligning gel images and matching protein spots across gels, to open the way toward the final quantification of spot intensities across all gel images. Once protein spots are matched and quantified, statistical methods can be applied to identify the relevant upregulation and downregulation.

The present volume is focused on deepening the analysis of 2-D maps by bioinformatics tools for what regards both the image analysis process to detect and quantify protein spots and the statistical analysis carried out to identify candidate biomarkers (i.e., spots upregulated or downregulated across samples).

Two main approaches to the analysis of 2-D maps images are available:

- The first involves a step of spot detection on each gel image, to provide a final list of spots present in all the investigated gels, each characterized by its volume: the final differential analysis is then performed on the spot volume dataset obtained.
- The second is instead based on the direct differential analysis of the 2-D maps images following a pixel-based procedure.

In the traditional approach spot boundaries are identified on each gel and spots are matched across multiple gels using a reference or master gel. However, this approach suffers from an important drawback: when the matching method fails, missing values are introduced in the spot volume data table; they can be due to the absence of a spot on the gel (in this case the missing value is a true zero) or to a failure in matching (in this case instead the missing value cannot be substituted by zero). Another problem regards the

definition of spot boundaries, which is particularly challenging in the case of overlapping spots. The pixel-based approach can overcome these problems, but both approaches rely on proper gel alignment; moreover, the pixel-based method is computationally intensive.

The volume is structured in four parts. The first part is devoted to deepening the problem of 2-D maps reproducibility and maps modeling. The validity of the results obtained by the final differential analysis deeply depends on the choices made during the experimental planning. This aspect is addressed in the first part of the book, to provide general good practices for a correct experimental design. In this part, the problem of spot overlapping is also addressed, and the main software packages available for 2-D maps analysis are presented.

The second part instead is devoted to spot-based methods: algorithms for maps denoising, background removal, and normalization are presented; the problem of image warping and spot detection and matching are presented and the most widespread algorithms available are described in detail.

The third part is mainly devoted to the description of classical and multivariate statistical methods that can be applied to spot volume datasets for the identification of candidate biomarkers.

The last part finally is focused on direct image analysis tools through pixel-based approaches.

The mathematical and statistical procedures are described from a theoretical point of view, to provide the basis for their correct applications, but examples of applications are also provided. The book is in fact thought to be of use for both the insiders of 2-D map image analysis and the researchers exploiting 2-D maps analysis in a wizard-like procedure: to the first ones the book is intended to as a compendium of the most recent applications, while for the second ones as a guide to help in the understanding of all the main steps of image analysis, to avoid errors and misinterpretations during the image analysis process.

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