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## A NOVEL GAUSSIAN FITTING APPROACH FOR 2D GEL ELECTROPHORESIS SATURATED PROTEIN SPOTS

Massimo Natale<sup>1,2</sup>, Alfonso Caiazzo<sup>3</sup>, Enrico M. Bucci<sup>2,4</sup> and Elisa Ficarra<sup>1</sup>

<sup>1</sup>*Department of Control and Computer Engineering, Politecnico di Torino, Torino, Italy*

<sup>1</sup>*BioDigitalValley srl, via Carlo Viola 78, Pont Saint Martin (AO)*

<sup>3</sup>*WIAS Berlin, Mohrenstrasse 39, 10997 Berlin, Germany.*

<sup>4</sup>*Istituto di Biostrutture e Bioimmagini, Via Mezzocannone 16, 80134, Naples, Italy*

*{massimo.natale, elisa.ficarra}@polito.it, caiazzo@wias-berlin.de, enrico.bucci@biodigitalvalley.com*

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**Abstract:** Analysis of 2D-GE images is a hot topic in bioinformatics research, since currently available commercial and academic software has proven to be not really effective and not completely automatic, often requiring manual revision of spots detection and refinement of computer generated matches. In this work, we present an effective technique for the detection and the reconstruction of over-saturated protein spots. Firstly, it reveals overexposed areas where spots may be truncated, and plateau regions caused by smeared and overlapped spots. As next, the correct distribution of pixel values in the overexposed areas and plateau regions is recovered by a two-dimensional fitting based on a generalized Gaussian distribution approximating the spots volume. Pixel correction according to the generalized Gaussian curve in saturated and smeared spots allows more accurate quantifications, providing more reliable image analysis results. As validation, we process highly exposed 2D-GE image, containing saturate spots, with respect to the corresponding non-saturated image, confirming that the method can effectively fix the saturated spots and enable correct spots quantification.

### 1 INTRODUCTION

In the post-genomic era, two-dimensional gel electrophoresis (2D-GE) is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples. In 2D-GE proteins are separated according to their charge (pI) by isoelectric focusing in the first dimension, and according to their molecular weight

(MW) by SDS-PAGE in the second dimension.

Each resulting 2D-GE contains a few hundred up to several thousands of protein spots whose volume correlated with the protein expression in the sample. The main goal of comparative proteomics is to match protein spots between gels and define differences in the expression level of proteins at different biological states using image analysis software.

Good image capture is critical to guarantee optimal performance of automated image analysis packages and generate reliable scientific data. It should allow for detection from very low to high abundant protein amounts. Through digitalization, a gel is represented by a two-dimensional matrix of squares or pixels. Each pixel of the generated image is defined by its coordinates  $(x,y)$  and by its signal intensity  $I$  encoded as greyscale level. The spatial coordinates are defined by image resolution, while the signal intensity is defined by dynamic range.

When optimising the dynamic range, it is important to avoid saturation effects. Saturation occurs when grey levels exceed the maximum representable. When a spot becomes saturated, the spot appears truncated when viewed in 3D, and any differences in high pixel intensities cannot be resolved because the missing area of the spot will not be measured. No reliable quantitative data will be generated from a saturated spot, and different authors recommend to manually deleting the saturated spots, before analyzing the gels with the available software (Berth, 2007). Where different experimental states are being compared the inclusion into the analysis of saturated spots have the potential to bias normalization, in particular if they have a variance that is a significant proportion of the total spot volume (Miller, 2006).

Currently available commercial software (as Delta2D, ImageMaster, PDQuest, Progenesis) are not able to deal with specific protein spot distortions found in the gel images (Maurer, 2006).

Automatic reconstruction of over saturated protein spots is impossible using available protein analysis software. So far, when an image has been over-saturated during scanning, there is no way to get the missing data back. The only option suggested by the producers of software for 2D-

GE analysis is to rescan the gel decreasing the exposure (Nonlinear, 2011). Most of the time, this is not possible because gel staining are photosensitive, and loss intensity in few minutes, or the image analysis is performed some time after the acquisition of the gels.

In this work, we present a novel algorithm for the detection and the reconstruction of over-saturated protein spots. Firstly, the algorithm reveals overexposed areas where spots may be truncated. Secondly, this method processes the distribution of the greyscale values in the saturated spots by a Gaussian mathematical model for the reconstruction of over-saturated protein spots.

Furthermore, the algorithm will be validated processing highly exposed 2D-GE image, containing saturate spots, to the corresponding non-saturated image.

## 2 METHODS AND RESULTS

### 2.1 Despeckle

After image scanning the 2D-GE are noisy, with artifacts as scratches, air bubbles, and spikes. The aim of reducing speckles in a 2D-GE image is to remove the noise without introducing any distortion in quantitative spot volume data. We evaluated filter size from  $3 \times 3$  to  $7 \times 7$  and it getting worse with the increase of filter size because the larger filter decreases the contrast of the image. However a  $3 \times 3$  filter is capable of removing most of the spikes and it has minor smoothing effects. The result of  $3 \times 3$  median filter is shown in figure 1. In figure 1A is shown a portion of gel where the spikes are marked with black arrows. In Figure 1C is shown the portion of gel in 3D view where a spikes is marked with a red arrow. In Figure 1B and 2D are shown the image and the 3D view after applying the  $3 \times 3$  median filter.

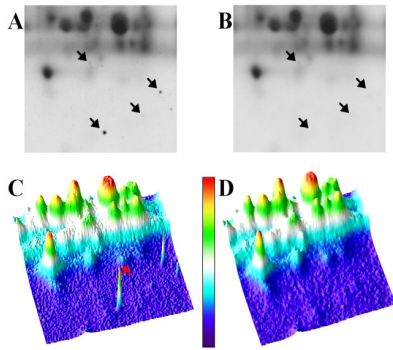


Figure 2: A) Portion of 2D-GE gel where the spikes are marked with black arrows. B) Portion of 2D-GE gel after the 3x3 median filter. C) , D) 3D view of the portion of gels

## 2.2 Find Plateau regions

The commercial software are able to detect a saturated area when a region of the image reaches the maximum value of greyscale. However in most cases a saturated area looks like a plateau zone, where the pixels have similar intensity, but they do not reach the maximum value of greyscale.

In these cases the commercial software not only are unable to correct this aberration but they also fail to detect it, inducing the operator to underestimate the problem.

In order to identify plateau regions, we implemented a morphological filter that allows to segment the plateau zones, inspired by the Rolling Ball algorithm described by Sternberg (Sternberg, 1983). We have developed the structural elements as a ball which has a circular shape placed onto the image defined by radius (RD) and the height defined by greyscale value tolerance (GVT). The RD and the GVT are defined by a single parameter. The RD represents the number of pixels of the circle radius which is used as structural elements (Figure 2A). The GVT represents the rate of gray values of the pixel in the centre of the structural elements (Figure 2B).

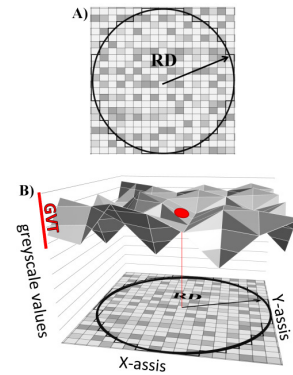


Figure 1: A) The radius defines the pixels that are included into the structural element. B) The greyscale value tolerance defines the variation within the gray values are considered to plateau.

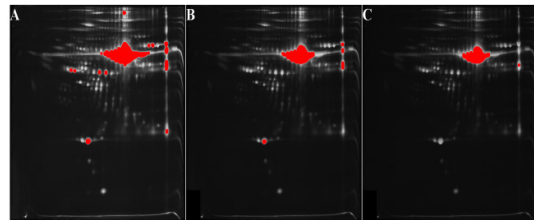


Figure 3 : saturation zone detected from our algorithm. The figure shows the same gel acquired with three different exposure. 2D-GE gel acquired with A) high, B) medium, and C) low exposure.

For example when the operator sets 10 as parameter, the RD is 10 pixels and the GVT in the 10% of gray values of the pixel in (x,y) position.

The centre of the structural element is moved along each pixel of the image and for each point (x,y) the maximum and minimum gray value of the pixels within the given RD is calculated. When the difference between maximum and minimum is less than the GVT, the area defined by local operator is considered as a plateau area.

When the parameter has low values the RD of the ball is small, so few pixel are included in the structural element, while the difference from maximum and minimum level must be small.

In order to test our plateau area finder algorithm, we performed the method on more than 50 2D-GE images, each acquired at 3 different expositions. An example is shown in figures 3A, 3B and 3C. The plateau areas found by our algorithm are filled in red. In particular, figure 3A contains a larger number of spots, but some of them are saturated. In this case, only image in figure 3C could be correctly analyzed by available software. The other two images would be discarded because of the large saturated areas that prevent accurate protein expression measures.

However, in several cases researchers would be interested in analyzing the image acquired with the largest exposure (figure 3A), which also contains the highest number of spots (and thus also the lower-abundance protein spots). For this purpose we developed a refined algorithm capable of calculating the original non-saturated distribution of the gray values within the saturated zone of the spot.

### 2.3 Gaussian Fitting

To determine the unknown distribution of gray values in the saturated area, we firstly assumed that the intensity values distribution in the spot is described by a Gaussian function of the form (1):

$$f(x, \sigma, M) = \frac{M}{\sqrt{2\pi}\sigma} \exp\left(-\frac{x^2}{2\sigma^2}\right) \quad (1)$$

where  $\sigma$  and  $M$  are, respectively, the standard deviation and the average of intensity values, while  $x$  is a pixel coordinate in the image. To determine  $\sigma$  and  $M$ , we search the function in the form (1), which fits at best the values of the unsaturated spot. In other words, given a set of  $m$  points with coordinates  $(x_j, y_j)$ , for  $j = 1, \dots, m$ , the problem is to find the couple of parameters  $(\sigma, M)$ , such that the differences (2)

$$f(x_j, \sigma, M) - I_j \quad (2)$$

are small, where  $I$  is the pixel intensity. The goodness of the approximation of the resulting fitting can be defined in different way (according to different norms).

Choosing the approximation criterion is equivalent to define an error function, describing how far is the Gaussian approximation from the original dataset in the unsaturated region, which must be minimized. For example, it can be considered to sum the squared differences, which gives the error function:

$$E(\sigma, M) = \sum_{j=1}^m (f(x_j, \sigma, M) - z_j)^2 \quad (3)$$

Other possibilities consist in taking different powers of the differences, or including a set of weights, e.g. to give different relevance to the point closer to the saturation. Finally, the problem is then reduced to find the couple  $(\sigma, M)$  giving the *minimum* of the selected error function. A possible approach is to find the minimum by an exhaustive search on the values of the parameters. If the size of the parameter space is too big, a more effective Newton-Raphson algorithm to find the zero of the gradient can be employed.

In figure 3 we showed that the same gel can be purchased with or without saturated spots (Figure 3). These gels provide us the opportunity to see how the gray values are distributed in the same spot.

In figure 4 we show the distribution of gray values in non saturated spots (figure 4A) and in saturated spots (figure 4B). The figure 4C and 4D show the intensity distribution profiles. In figure 4B and 4D are underlined the saturated value by red lines.

In figure 5A we show as our mathematical model, the Gaussian function (1) where the parameters have been obtained through

the minimization of (3), is able to accurately describe the distribution of values of a 2D-GE spot.

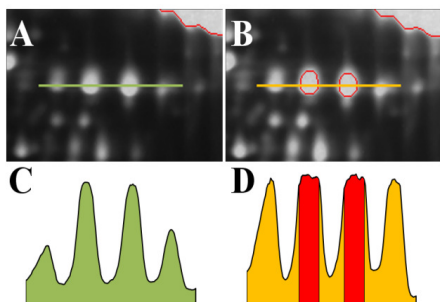


Figure 4: A) Portion of 2D-GE where all spots are correctly acquired. B) Portion of 2D-GE where two spots are saturated. C) Plot profile of gray value found along the green line as shown in 4A). D) Plot profile of gray value found along the yellow line as shown in 4B). In red are shown the saturated zone.

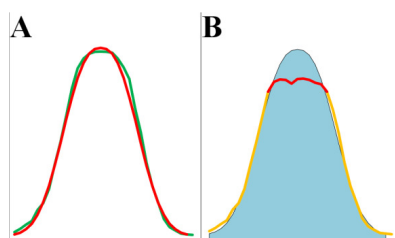


Figure 5: A) In red is plotted the Gaussian distribution, in green is plotted the spot non-saturated profile. B) In blue is plotted the Gaussian distribution while in yellow is plotted the spot saturated spot.

Finally, in figure 5B we show as a demonstration that the method can effectively fix the saturated spots and enable correct spots quantification.

### 3 CONCLUSIONS

Saturation of abundant spots is a general problem in 2-DE evaluation, in particular working with complex samples like serum or plasma, which have a very uneven protein distribution. Currently available commercial software are not able to perform 2D-GE image analysis in presence of saturated spots. The only alternatives are to

remove the saturated spots or rescan the gel image with different acquisition parameters.

In this paper is presented a new approach for treatment of over-saturated protein spots in 2D-GE.

Using experimental 2D-GE images we have demonstrated that saturated protein spots can be found by our algorithm.

Subsequently, we applied a Gaussian function to calculate the real experimental spot volume (and thus the correct protein expression) through the reconstruction of intensity distribution of non-saturated spots. The accuracy of reconstruction was verified by comparing the same gel acquired with or without saturated spots. The proposed technique has been shown to be able to restore the spot volume.

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