INTRODUCTION

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.

RESULTS

Figure 1

In order to identify plateau regions, we implemented a morphological filter inspired by the Rolling Ball algorithm (Sternberg, 1983). We designed structural elements as ball of circular shape with radius (RD) and height defined by grayscale value tolerance (GVT). The RD represents the number of pixels of the circle radius (Figure 1A). The GVT represents the rate of gray values of the pixel in the centre of the structural elements (Figure 1B).

Figure 3

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):

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

(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 optimal fitting of the Gaussian function in (1) with the values of the unsaturated spot. In other words, given a set of \( m \) pixels with coordinates \( (x_j, y_j) \), for \( j = 1, \ldots, m \), the problem is to find the couple of parameters \( (\sigma, M) \) such that the differences (2):

\[ f(x_j, \sigma, M) - I_j \]  

(2)

are small, where \( I \) is the pixel intensity. 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. As error function we selected the sum of the squared differences:

\[ E(\sigma, M) = \sum_{j=1}^{m} (f(x_j, \sigma, M) - I_j)^2 \]  

(3)

Finally, 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.