

COMPENSATION OF SMILE EFFECT DISTORTION IN ELECTROPHORETIC GEL IMAGE

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Abstract: This paper is engaged in the issue of automatic detection and removal of smile effect geometrical distortion in agarose gel electrophoresis images. Based on created databank of electrophoretic phantoms, an algorithm that is able to repair mentioned smile effect distortion was created. In this paper, two gel images with applied removal algorithm are shown with percentage description of reparation level.

Keywords: agarose, electrophoresis, distortions, smile effect, reparation

1. INTRODUCTION

Gel electrophoresis is a widely used separation method, using applied electric field for migration and separation of differentially charged molecules by their charge and molecule size. This method, even though it is quite archaic (first electrophoretic aperture dates back in 1937) is commonly used in immunology, biology and forensic criminology, which emphasizes solid separated sample recognition for the most accurate classification [1].

Due to wide usage of gel electrophoresis in this day and age, many researches have emerged, to provide software for automatic gel analysis, which would be able to evaluate the fragment size from each electrophoretic run based on band position in each lane given [2].

Unfortunately, in many gels, smile effect distortion, caused by application of high voltages is present, which reduces the chances of successful band size recognition and analysis.

1.1. SMILE EFFECT

Smile effect is mainly caused by the usage of high applied voltage, according to experiments, higher than 150V. Injected samples then run with different electrophoretic mobility in different parts of gel with different temperatures, causing the smile- or frown-shaped distortion, affecting the whole gel. This distortion can also be caused by low concentration of agarose in gel carrier and by applying usual voltage values [4].

1.2. TESTED DATA

Since there is no reliable database of gel electrophoresis images with described effect of smile effect distortion, electrophoretic gel phantoms were created to simulate a wide spread of geometric distortions, caused by artificially created errors. Phantoms, containing smile effect, were used for modeling, testing and analysis of smile effect distortion correction methods.

2. DISTORTION REMOVAL APPROACH

In the first step, the input phantom image is visually enhanced by averaging filters, spur pixels removal and contrast transformation. Individual lanes, containing bands, are then found by iterating through every vertical image line and by finding vertical lines with no dark pixels present. These

vertical lines are creating boundaries between lanes containing bands and empty space in the image, so they can be used to find approximate middle points in every lane containing bands. Vertical line, passing through this middle point, is then extracted, and lowest band present (smallest one) is detected in every lane by exceeding the set threshold. These end bands are then fitted with fourth-grade polynomial curve (Fig. 1). The extracted equation, describing the curve, gives the information of vertical axis (y) ascension or descent of x -th pixel. If given pixel ascends, created buffer A indexes integer i for the x -th position, if the pixel descends, it indexes integer $-i$. Buffer A then can be multiplied by integer constant to regulate the power of resampling, or some positions can be nulled for smoothening the resampled image bottom edge.

In the next step, the resampling process takes place, and every x -th vertical lane is either stretched or shortened, based on the increasing or reduction of resampling ratio by adding $A(x)$ integer to this ratio. After this process takes place, the character of smile effect deformation is corrected [3].

Before and after the resampling process, band positions in the first lane are detected by thresholding in the vertical line, passing through middle point in the first lane, which contains bands. These band positions are fitted with a polynomial curve along with corresponding band sizes and this curve can then be used for interpolating kilobase size values for band positions in every other lane, detected the same way. With the knowledge of band sizes before (Be) and after (Af) resampling process, and with the information of correct ladder band sizes ($ladder$), percentage of correction success can then be calculated by given formula (1), where v is band vertical order, and z is lane horizontal order.

$$\text{percentage of correction succes} = 100 * (\text{abs}(Be(v, z) - Af(v, z)) / \text{ladder}(v)) \quad (1)$$

3. RESULTS AND DISCUSSION

To show the success of the created smile effect removal algorithm, two gel images were processed in Matlab environment by the described removal algorithm.

The gel No. 1 show signs of smile effect distortion, causing the bands in all lanes to be in different positions. Chosen algorithm has successfully resampled most of the bands into almost the same vertical height, as the bands in the left reference lane (Fig. 1), therefore the algorithm usage results in the percentage of reparation in every base pairs size category, summed up in Table 1.

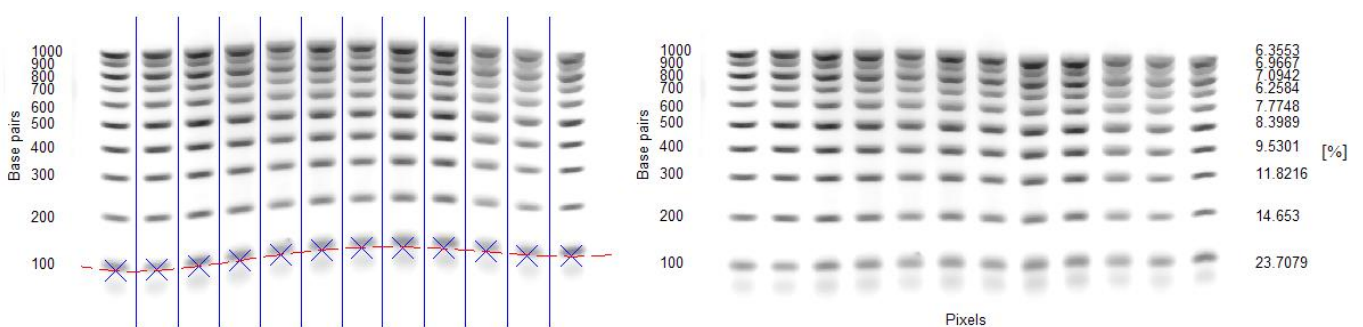


Figure 1: Results of smile effect distortion correction in every band size, image No. 1

Band size [bp]	1000	900	800	700	600	500	400	300	200	100
Correction [%] Im. No. 1	6.36	6.97	7.10	6.26	7.77	8.40	9.53	11.82	14.65	23.71

Table 1: Percentage of correction success in different band sizes of images

To apply this principle on a real electrophoretic images, the bank of buffers *A* has to be created, and the choice of needed buffer can be then given to an application user, to choose the most fitting polynomial curve subjectively, or by algorithm, that measures, for example, the slope of last bands in the first and in the last lane of the image. As an example of the approach applied on a real image, subjectively the best possible fit for *A* buffer creation was used, and one real image was then resampled by given *A* buffer (Fig. 2).

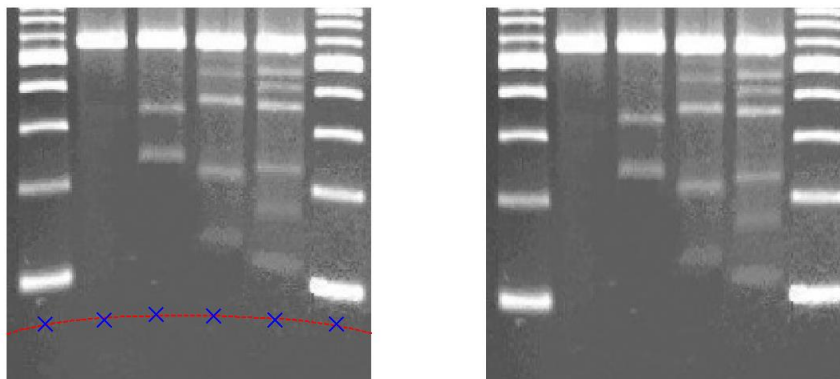


Figure 2: Real image after application of correction algorithm

4. CONCLUSION

The recent need of fast analysis of all the available results in every scientific field requires non-distorted and non-damaged data. This need arises even in the gel electrophoresis classification, and that is why it is important to reduce as much distortions as possible. This paper shows created approach for removal of smile effect distortion in agarose gel electrophoresis. Results of elimination are shown in two gel images. The average correction percentage in phantom gel image is 10.26 %. To apply created principle on a real gel images, the creation of bank of either derived or manually arranged *A* buffers can be created, from which the most ideal buffer can be chosen (Fig. 2).

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