Improved Accuracy of Band Detection in GASepo System for Quantitative Analysis of Images in Epo Doping Control

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Abstract: *In the year 2003, World Anti-Doping Agency (WADA) initiated an international project with the goal to develop a suitable and easy to use software system aimed at the standardized quantitative analysis of digital images acquired during the process of erythropoietin (Epo) doping control. Within the cooperation between Austrian Research Centers GmbH and Institute of Measurement Science, Slovak Academy of Sciences, improved image preprocessing method has been developed. The proposed method – Band Straightening Algorithm (BSA) – significantly reduces local geometric distortions in Epo images that cause band detection problems, especially incases with geometrically distorted and disrupted bands. The accuracy of the band detection is one of the most critical points of the Epo image analysis. In the year 2006, the proposed method has been implemented in the latest release of the software package GASepo 2.1, which is used in Doping-Control laboratories worldwide.*

Keywords: Epo doping control, image segmentation, band detection, image filtering, geometrical distortion correction, correlation analysis

1. Introduction

In the last decade, the synthesis of recombinant forms of human peptide hormone erythropoietin (Epo) became possible due to advances of molecular engineering. This substance was originally intended for therapy of diseases associated with lack of endogenous human Epo which controls the production of red blood cells. In a short time the recombinant erythropoietin (rEpo) turned to be a popular doping substance especially in endurance sports where performance boost of up to 10% can be achieved [1,2]. Unlike the conventional doping substances (e.g. anabolic steroids) rEpo cannot be detected by conventional mass-spectroscopy methods [4].

The research has shown that the difference between rEpo and native human Epo is hardly evident as late as in post-translational phases in urine of a doped individual. Detection of such small differences sis done by double-blotting method based on isoelectric focusing in the gradient of pH in polyacrylamide gel [4]. Afterwards, Epo isoforms are made visible by a chemoluminescence reaction and recorded by a standard analog or digital photographic technique (see Fig. 1).

In Austrian Research Centers, the unique software package GASepo (Gel Analysis System for Erythropoietin) has been developed and put into the praxis in many WADA accredited doping control laboratories.

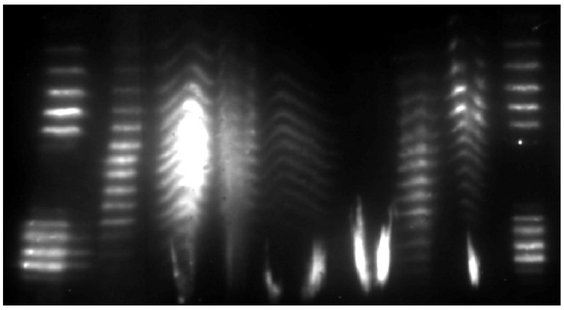

Fig. 1. The example of an Epo image where the global and local geometric distortions are present. Leftmost and rightmost lanes represent samples of the doping standards EPOα,β and DARBα. All other lanes represent samples taken from tested individuals.

2. Manual image rectification in GASepo

Geometric image distortions are the most often reasons of unwanted output errors. Therefore, it is essential to rectify the most striking geometric distortions present in Epo images before any further image analyses. The global image distortions have to be corrected manually requiring interactive intervention of the user (see Fig. 2 – left image). Manual corrections of the local image distortions are usually too time demanding and therefore unable to use (see Fig. 2 – right image).

3. Epo doping positivity criterion

The main task of a doping positivity decision is to find a right placements of two reference cut-off lines that separate the groups of EPOα,β and DARBα bands from the remaining bands reflecting natural Epo (see Fig. 3). The final doping positivity criterion counts with exact positions of all bands that are compared with the positions of cut-off lines.

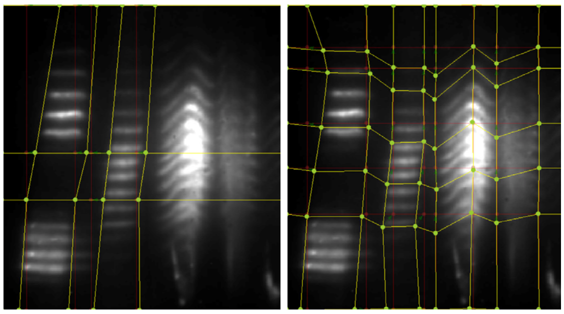

Fig. 2. The example of the manual correction of the global (left) and local (right) image distortions in *GASepo*.

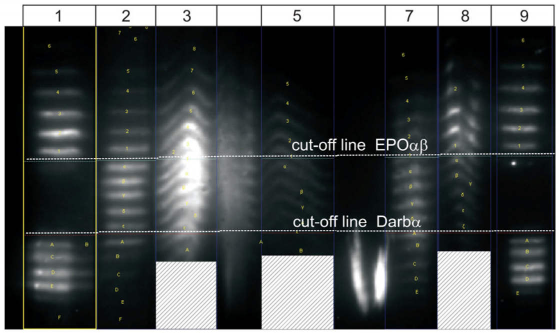

Fig. 3. The result of the initial part of an Epo image analysis. The two reference cut-off lines are placed according to the doping standards (lanes 1 and 9).

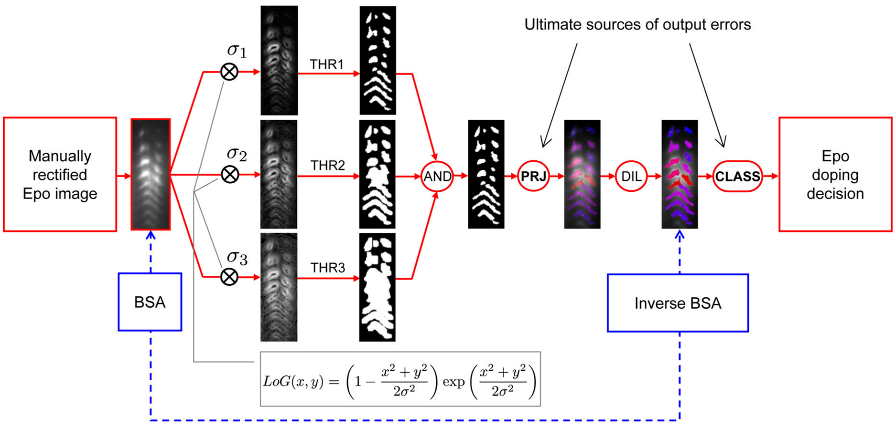

Fig. 4. The basic scheme of the band detection process. Schematic embedding of the new Band Straightening Algorithm (BSA) is marked with the blue color.

4. Epo image segmentation and classification

Due to the mentioned Epo doping positivity criterion, the crucial point of Epo image processing is the procedure of the band detection. This procedure consists of two main steps – image segmentation and object classification. The segmentation is a multistage process in which the original grayscale image is transformed into a binary map [5]. Detected objects are joined together in the projection operation (PRJ) and subsequently classified into two groups – band and artifacts [6] (see CLASS in Fig. 4). These operations are highly sensitive to quality of input images and therefore they introduce ultimate sources of output errors (see Fig. 5).

5. Band Straightening Algorithm (BSA)

For suppressing unwanted local geometric distortions present in Epo images, a new image preprocessing method has been developed. The main idea of Band Straightening Algorithm (BSA) is automatic search for an optimum vertical shifts of all image columns that lead to maximum reduction of the local geometric distortions (see Fig. 6).

The search is performed in two successive phases – the linear search followed by the gradient descent search. Both methods minimize the total row-wise tension of an input image. An optimum shift vector k_{opt} is calculated according to the following formulaes:

$$k_{opt} = \arg\max_{k} \left\{ M\left(\tilde{L}^k\right) \right\},$$

$$M\left(\tilde{L}^k\right) = -\sum_{i=1}^{m} Tens\left(\tilde{R}_i^k\right),$$

$$Tens\left(\tilde{R}_i^k\right) = \sum_{j=2}^{n} \left(\tilde{a}_{i,j} - \tilde{a}_{i,(j-1)}\right)^2,$$

$$\tilde{R}_i^k = \left[\tilde{a}_{i,1}, \tilde{a}_{i,2}, \ldots, \tilde{a}_{i,n}\right]$$

Fig. 5. The example of an incorrectly segmented Epo image in consequence of strong local geometric distortions. One can see the objects consisting of wrong object fragments joined by the confused projection operation. The joined objects are shown in the same color index. The desired correct objects are bordered by the solid line.

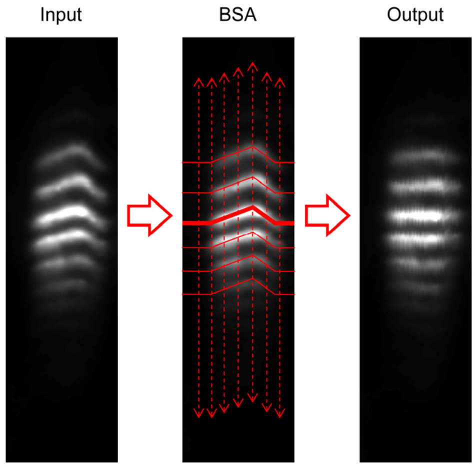

Fig. 6. Basic principle of BSA schematically explained on a model Epo image. Local geometric distortions are being reduced by the automatic search for an optimum vertical shifts of all image columns.

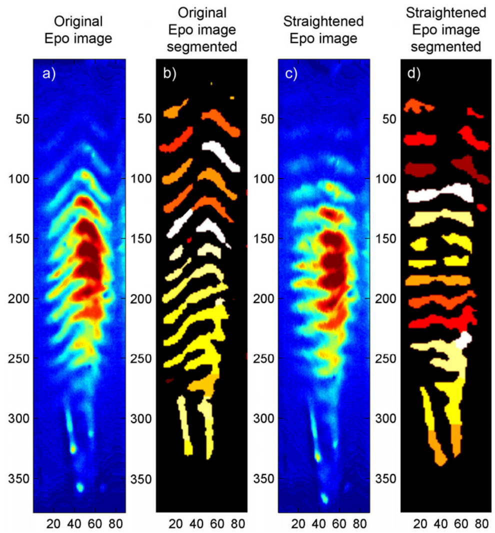

Fig. 7. The example of an Epo image processed without (a, b) and with (c, d) the use of BSA. One can see the clear straightening effect of the source image using BSA (c vs. a) as well as the remarkable improvement of correct merging of related object fragments (d vs. b).

where $\widetilde{L}^k$ is the shifted Epo image by a shift vector k, $M\left(\widetilde{L}^k\right)$ is the maximized cost function, $Tens\left(\widetilde{R}_i^k\right)$ is the row tension of the i-th row $\widetilde{R}_i^k$. The detailed description of the algorithm is given in [8, 9].

This approach provides substantial improvement of image quality on acceptable computational complexity level. The experiments with real Epo data have shown that BSA is capable of lowering average classification error up to 2-4 times in computational time $O(mn^3)$, where m and n are height and width of processed image (see Fig. 7).

6. BSA implementation in GASepo

In 2006, the novel BSA method was successfully implemented in the latest release of the software package GASepo 2.1 (Fig. 8). This implementation required: first, to prepare a prototype of BSA and its careful testing in Matlab environment, then its optimization in Microsoft Visual C# 2005 taking advantages of Microsoft .NET Framework 2.0.

7. Conclusions

Since a number of Epo images suffer from geometric distortions which affect relevant operations applied to them in GASepo software, a new image preprocessing method – Band Straightening Algorithm – has been developed. This method enables substantial reduction of the local geometric distortions resulting in the decrease of average classification error by up to 2-4 times whereby the computational complexity stays on acceptable level – maximum time $O(mn^3)$. In 2006, the proposed method has been implemented in the latest release of the software package GASepo 2.1, which is used in DC labs worldwide.

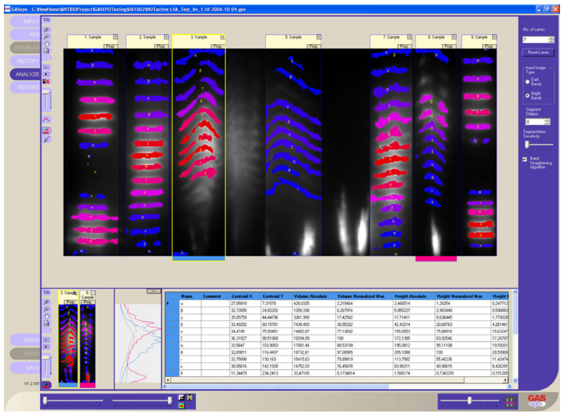

Fig. 8. Screenshot of the new *GASepo 2.1*.

8. Acknowledgements

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9. References

[1] Bajla I. et al.: Analysis of EPO Images after Isoelectric Focusing and Double Blotting. In: Proceedings of the Second Intern. Conference on Biomedical Engineering *IASTED* 2004, 16–18 February 2004, Innsbruck, Austria, p. 228–233.

[2] Bajla I. et al.: GASepo – a software solution for quantitative analysis of digital images in Epo doping control. *Computer Methods and Programs in Biomedicine* 80(3), 2005, p. 246–270.

[3] Holländer I. et al.: GASepo – System for Analysis of Images Generated in Epo Doping-Control. In: Proceedings of the 17th Biennal Intern. EURASIP Conference *Biosignal* 2004, 22–26 June 2004, Brno, Czech Republic, VUTIUM Press, p. 273–277.

[4] Lasne F., de Ceaurriz J.: Recombinant erythropoietin in urine. *Nature*, 2000, p. 405-435.

[5] Ramoser H. et al.: Segmentation of Electro-phoretic Images in Doping Control. In: Proceedings of the Intern.

Conference on Mathematics and Engineering Techniques in Medicine and Biological Sciences *METMBS* 2004, 15–16 June 2004, Las Vegas, USA, CSREA Press, p. 467–470.

[6] Witkovský V. et al.: Alternative Approaches to Band Classification in Epo images for the GASepo software. Technical report ARC–IT–0115, January 2005, ARC Seibersdorf research GmbH, 60 pp.

[7] Štolc S., Bajla I.: Improvement of band segmentation in Epo images via column shift transformation with cost functions. *Medical and Biological Engineering and Computing* 44 (4), 2006, p. 257-274.

[8] Štolc S., Bajla I.: Improvement of band segmentation in Epo images via column shift transformation with cost functions (Erratum). *Medical and Biological Engineering and Computing* 44 (7), 2006, p. 605-607.