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GASepo: System for Analysis of Images Generated in EPO Doping-Control Proteomics

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Introduction

Human Erythropoietin (hEPO) is a glycoprotein hormone, which is mainly produced by the kidneys in adult humans. Erythropoietin acts as a mediator of hematopoiesis and therefore is mainly involved in red blood cell development and regulation of oxygen supply. Renal dysfunction leads to severe forms of chronic anemia and hypoxia. Recombinant human EPO (rhEPO) was the first effective pharmaceutical product for treatment of anemic patients. Beside their vital therapeutic importance rhEPO and its derivatives (e.g. NeoRecormon, Erypo, Aranesp) became increasingly misused for improving endurance performance in sports. The International Olympic Committee (IOC) therefore banned rhEPO administration in 1990. Recently a method based on the separation of rhEPO isoforms from human urine using isoelectric focussing (IEF) was developed by Lasne(1-4) for the direct detection of rhEPO abuse.

Based on this proteomic approach a novel bioinformatic platform for gel image processing called GASepo was developed and applied to EPO doping control at our laboratories.

Basic Image Processing Steps in GASepo

Image processing in GASepo is basically divided into the following four steps:

1. Image Input: An EPO image acquired by either a charge-coupled device (CCD) camera or a film scanner is read into the system in a broad choice of image formats. The region of interest is chosen interactively and the system automatically suggests image division into individual lanes.

2. Noise Suppression: Special image processing algorithms originated in the magnetic resonance (MR) tomography (based on so-called Geometry-driven diffusion methods) are used

to suppress image noise without affecting (smoothing) the object boundaries. This noise reduction method is particularly suited for subsequent segmentation.

3. Background Correction: An original two-dimensional algorithm is used to correct the additive background of the images. The left and right margins of the given lane are used as indicators of the background distortion and the correction surface is then computed by interpolation.

4. Segmentation: Individual lane images are segmented using original digital image segmentation methods. The segmented objects (bands) are then numerically evaluated.

Figure 1 shows a typical screenshot of the GASepo software.

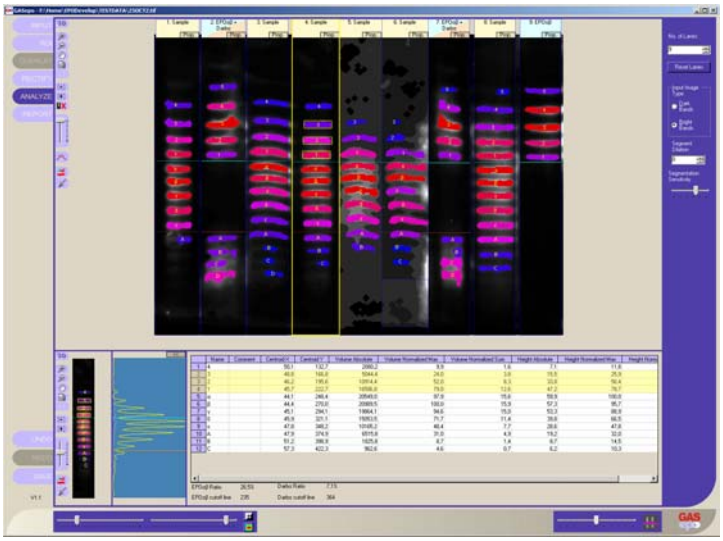

Figure 1. A screenshot of GASepo.

Band Segmentation and Classification: The Novelty in GASepo

Bands as Individual Objects: The positivity criteria agreed upon by a World Anti-Doping Agency (WADA) committee are based on analysis of individual bands in the relevant lanes. GASepo implements a digital image segmentation method: The goal of the method is to identify a band as a two-dimensional object.

The Segmentation Method: Despite the correction of most degradation effects (such as additive noise) in the preprocessing stage, the application of standard edge detectors or adaptive thresholding to band segmentation does not yield satisfactory results in EPO images. Therefore,

a band segmentation method has been developed which can be described as a sequence of operations specifically designed to solve the following crucial cases of band degradation: (i) blurred bands, (ii) bands which are merged into one blob object, and (iii) bands which have been disintegrated into multiple individual objects.

Object Classification: The segmentation procedure itself is based on grayscale information in the images and only takes into account the object/background contrast and the relative size of the object. The set of objects segmented in this way still includes a lot of artefacts (gel impurities or other defects). Therefore, segmented objects have to be classified according to their shape. We analyzed the geometry of the common bands and designed some geometry measures which describe the form and placement of an object. The decision criteria based on these measures have then been implemented as band/artefact classifiers.

Comparison with Expert Approach: To assess the quality of the segmentation procedure, we carried out an expert session: 112 lanes were segmented by one to three experts. 90% of all expert segmented bands are correctly detected by the automatic segmentation algorithm. The average distance between the centroids of corresponding bands is 2.23 pixels, the size of segmented bands is 70% of the corresponding expert segmented band. This uniform volume reduction can be easily corrected by dilation.

Interactive Tools: The GASepo software is equipped with a sophisticated support of the band segmentation. In addition to the features mentioned above, the user has the option of the band detection (this is a trade-off: more sensitive detection is less specific) as well as of the bands. All tools affect the whole image uniformly so the relative size of the band to each other is always maintained. The individual bands are interactively selectable and appear in tabular form on the screen together with their properties such as absolute and relative peak height, absolute and relative volume, centroid position etc.

Other GASepo Features

pH Reference Image: The software includes an option to combine the EPO image with a photography of the gel with some added to the catholyte. The photography of the gel (called “pH reference”) is taken at the end of the run in colour. The deformation of the methyl red line (which, ideally, should be straight) reflects the local deformations of the gel caused by pH gradient inhomogeneities (mainly due to high concentrations of urinary proteins). By superimposing this pH reference image with the EPO image it is much easier to identify the respective EPO bands in various lanes.

Automated Cut-Off Line Detection: We developed a novel method for automated detection of the cut-off line position, which is robust and reproducible within a broad gel quality range. A patent application for this methods has been submitted.

3D Display: Fully interactive three-dimensional (3D) display of the EPO image allows to detect even very weak bands.

Conclusion

The GASepo software has been developed in the framework of a WADA project which involves nine doping control laboratories worldwide. The mathematical background includes methods of image processing, fuzzy logic for object classification, cut-off line detection and others, as well as the system implementation (5). The partners act as software testers and consultants. The software will be distributed for free to all WADA-accredited laboratories with the aim to harmonize and standardize EPO analysis. It was successfully deployed, among others, during the Olympic Games 2004 in Athens and during the Winter Olympic Games 2006 in Torino. The entire project is planned for the period 2004–2006. The authors are working on further improvement, especially on automating the image analysis process.

For more details on the project and for software download, visit www.antidoping.at/epo .

Acknowledgement

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