Semi-Automatic Karyotype Generation System "Chromosomes"

Nenad V. Ilijic[1], Nemanja B. Grujic[2], Dragan Jankovic[3]

Abstract – **This work points out the problems that occur during the manual karyotype generation. A short explanation of medical statistical data and implications of this problem is included. Software package using new segmentation algorithm is presented. It presents the tool for semi-automatic karyotype generation. The main functionalities of package are presented.**

Keywords – **Karyotype, segmentation algorithm.**

I. INTRODUCTION

There are well known software packages for the karyotype generation in the market [5], [6]. They are characterized by very high market price. The goal of this project is to create a system which will significantly speed up the karyotype generation process and contribute to early detection of genetic malformations in prenatal diagnostics. Karyotype generation system should enable faster karyotype determining process without decreasing the flexibility a user has during the manual processing. This system is the result of karyotype generation and segmentation algorithm research made by the authors in past two years. A new segmentation algorithm is developed for purpose of karyotype generation. Importance of karyotype generation problem, and statistical data which implies it, are shown in the chapters II and III.

II. GOALS AND ACHIEVEMENTS

Number of anomalies in newborn children is increasing every year, which makes karyotype determining a very important procedure conducted by citogenetic laboratories. Citogenetic laboratories in our country perform this procedure manually. Some notable manual processing deficiencies are:

1. Extremely long image analysis process,
2. Increased error probability compared to application of specialized software packages,
3. Difficult data organization and categorization, especially when dealing with images in digital format.

The karyotype generation system is expected to facilitate:

1. Chromosome segmentation from preparation image.
2. Flexible manual manipulation of separated chromosomes.
3. Automatic generation of initial state of karyotype.
4. Possibility of simultaneous processing of several preparations.
5. Storing and printing of karyotype.
6. Differential diagnostic possibilities.

"Chromosomes" software package was developed in Microsoft Visual Studio .NET environment, using C# programming language and ActiveX technology, for Windows operating systems [3].

Focusing on solving afore mentioned problems, software package implements the following functionalities:

1. Workspace creation and reading of arbitrary number of digital images and their descriptive representation.
2. Application of automatic segmentation algorithm on the current image.
3. Manual chromosome separation using the available tools.
4. Automatic extraction of chromosomes from the resulting image which can be individually manipulated.
5. Automatic orientation and zooming of chromosomes.
6. Automatic karyotype proposition.
7. Manual classification of chromosomes into groups.
8. Free rotation and zooming of chromosomes.
9. Breakdown of groups of chromosomes into individual chromosomes.
10. Marking of non-chromosomal objects and their transfer to a list.
11. Restoration of chromosomes that were automatically removed.
12. Saving the entire workspace (input and output data) as a single project.

III. STATISTICAL DATA AND IMPLICATIONS

According to some statistical data, 2-4% or newborns has at least one chromosomal aberration in the karyotype, while in adults this percentage is lower 0.5-1%. This is because the newborns with chromosomal aberrations are less likely to reach adulthood. It is speculated that at least 7% of human zygotes has chromosomal aberrations, which would be the reason for a high percentage of spontaneous abortions. [1].

[1]Nenad V. Ilijic is with the Faculty of Electronic Engineering, Nikoletine Bursaca 16, 18000 Nis, Serbia and Montenegro, E-mail: ni@ulfserk.com

[2]Nemanja B. Grujic is with the Faculty of Electronic Engineering, Aleksinacka 28, 18000 Nis, Serbia and Montenegro, E-mail: jagru@bankerinter.net

[3]Dragan Janković is with the Faculty of Electronic Engineering, Aleksandra Medvedeva 14, 18000 Nis, Serbia and Montenegro, E-mail: gaga@elfak.ni.ac.yu

This statistics clearly implies the impact of genetic consultancies and software tools which will speed up the process of predicting possible malformations and make it a lot more efficient.

The role of genetic consultancy is to provide the patient and family members with information about type and nature of ailment, the development of ailment, possible consequences and therapies, mechanism of inheritance and possible prevention. Genetic consulting also provides risk estimates of the child being born with an inherited disease, as well as directing to prenatal diagnostics in cases where such risk is high.

This software package can be applied in several specific techniques used in prenatal diagnostics [2]:

1. Amniocentesis
2. Chorionic villi sampling
3. Citogenetic analysis
4. Cell culture in Vitro

IV. Program Functionality

"Chromosomes" software package satisfies several functional demands which give the advantage compared to the manual karyotype generation. Also, because of its modular build, it is easily upgradeable. The advantages of using this software solution are the following:

1. Faster image processing and karyotype generation.
2. Decreased error occurrence.
3. Organizing of data as projects.
4. Can be used as a starting point for the development of a more complex system (differential diagnostics methods).
5. With minor modifications, possible application in other areas where sample recognition plays a vital role (blood analysis).

Current level of functionality deals with organization of microscope acquired digital images as logical units (Workspaces), in order to achieve desired classification by responsible staff or patients. Each of the resulting images can be processed individually, so it is possible to store more processing results in one workspace (Figure 1). Once saved, a project is no longer depending on the source images, but is built in a workspace.

An automatic analysis can be performed on loaded images resulting in individual chromosomes (Figure 2). This analysis is achieved by use of several techniques for efficient chromosome separation. Input data is a digital image acquired from the microscope, and output data is an image with background and unwanted objects removed, as well as a list of chromosomes organized as individual projects.

Fig. 1. Working environment with several loaded images

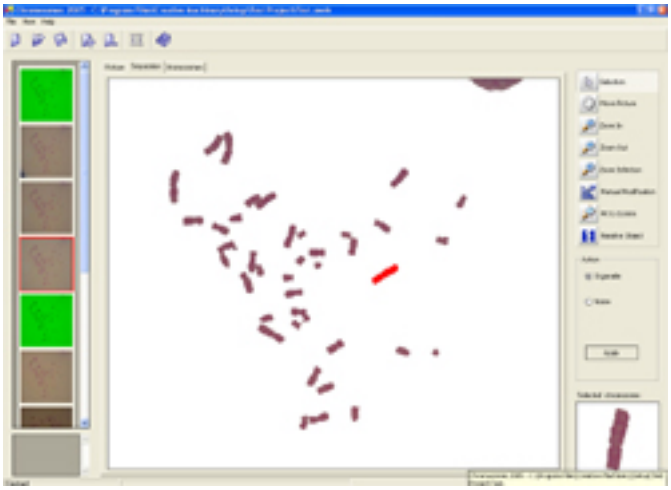

Fig. 2. Result of automatic separation applied on the previous example (Figure 1)

After the first pass all objects in the viewing area at the moment of image capture are extracted. There can be quite a lot of these unwanted objects, and coupled with equipment imperfections a consequence might be impossibility to place only chromosomes in the viewing area.

Among the chromosomes we can find:

1. Cell organelles,
2. Air bubbles,
3. Interference due to imperfections in optical equipment,
4. Local fluctuations in cytoplasm colour.

Because of this, during system implementation we must take into consideration the extraction of all these unwanted subjects. Irregularities, such as variable lighting can be seen on input images, which can make the differentiation of objects and background more difficult. Since there are more ways to colour the samples, the program must not make the difference between images made by different methods.

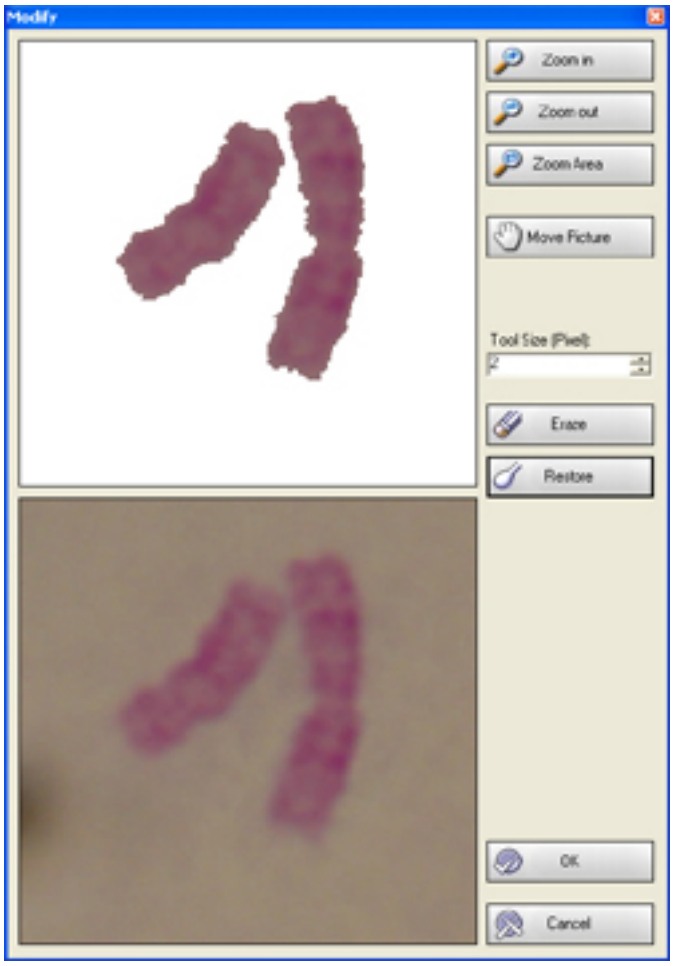

Fig. 3. Result of use of the tool for manual manipulation of digital images

Judging by the results of test samples, a chromosome is never removed as an unwanted object (noise); and the noise (either organelle or chromosome) is never marked as a valid object. In certain cases organelles similar in size and colour to a chromosome might be marked as a chromosome.

The biggest problem an algorithm might encounter is the overlapping or connection between the chromosomes, which results in those two chromosomes being extracted as a single unit; however an option of manual ungrouping is offered, and an automatic option is planned, too.

All unwanted phenomena can be removed by system of manual image manipulation. (Figures 3 and 4). User has two tools available, as well as several customary auxiliary tools:

1. Tool for removal of unwanted objects.
2. Tool for restoration of image parts that were removed by mistake.
3. Zooming tool (with centring or selection of a region to be magnified).
4. Image moving tool in order to focus the necessary part in the viewing area.

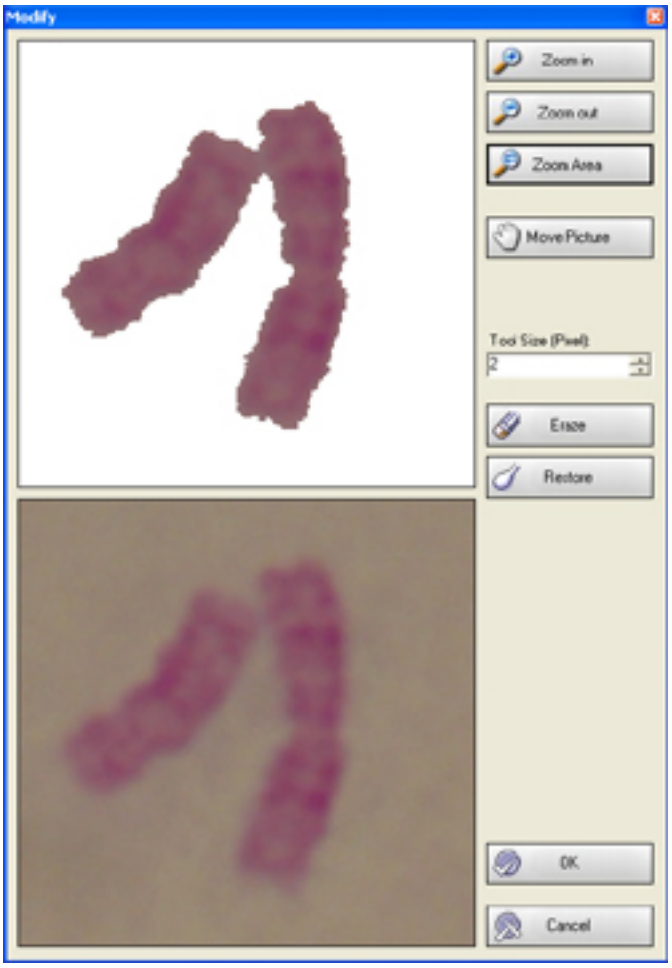

Fig. 4. Tool for manual manipulation of digital images

In order to facilitate this process, a user can work both on input and output image and user can copy contents from input image to an output image. All changes done in this manual image processing phase are reflected in the generated karyogram.

Next phase is work with output data acquired as a result of separation (both manual and automatic). The result might be:

1. Chromosomes,
2. Groups of chromosomes and
3. Possibly unwanted objects.

At the beginning of this phase all extracted objects are in the common list. Automatic karyotype proposal option is enabled, but this option makes sense only if the user is sure about the accuracy of the output results. Actually, this option should not be used if there are overlapped chromosomes and organelles in the list. Also, it is possible to manually assign chromosomes to the groups they belong to, by dragging from the common list of chromosomes to the corresponding group.

In the last phase of processing the user has several tools for manual processing of acquired data and upon their successful use the final list can be minimized to the array of chromosomes free of noise.

Those are:

1. Possibility of marking unwanted objects so they are treated as organelles or noise later on.
2. Separation of overlapped chromosomes.
3. Restoration of damaged chromosome parts.
4. Restoration of chromosomes that were marked as organelles or noise during the automatic separation phase.

Selected chromosome can be removed from the list of obtained chromosomes by being marked as an organelle or noise. That doesn't mean it's completely removed from the list of obtained objects, which can be reviewed at any time and the marked objects from it can be restored.

If there were overlapped chromosomes on the original image, they will be extracted as individual objects which can be separated into individual chromosomes in the last phase. This is done by the same means of manipulation that is being used in the manual processing of the entire output image. In the case of chromosomes being damaged by the algorithm, damaged parts of the chromosome can be restored from the original image.

Fig. 5. Result of automatic karyotype proposal on separated chromosomes

As mentioned above, after the generation of the final list of chromosomes, it is possible to manually assign chromosomes to the groups they belong to, but it is also possible to apply the automatic karyotype proposal option (Figure 5).

We can perform rotation and scaling of the extracted chromosomes, and those actions can be performed locally (on one chromosome) or globally (on all chromosomes).

Algorithm will automatically attempt to orientate all chromosomes vertically and to maintain their relative proportions (by magnifying them relative to the largest chromosome).

V. Conclusion

The problem of automatic karyotype generation, and especially segmentation of chromosomes from a digital image, is a very complex problem which requires practically years of work and efforts by whole teams of experts [4]. This area is still open for research and there is still no complete and fully functional solution for this problem, based on undefined nature of object recognition in general. "Chromosomes" software package should be a step forward towards the more efficient resolution of this problem. It yields results with monochromatic input images, meaning that it can be used without the need for expensive aparatus used for multispectral coloring of chromosomes. Since it's organized as a completely functional environment it is suitable for work in healthcare establishments and also commercial application.

References

[1] *Stevo Najman*, OSNOVE MOLEKULARNE I HUMANE GENETIKE, Niš, Savez studenata medicinskog fakulteta, 2002.

[2] *Živojin Stanković, Jelena Živanov-Ćurlis, Stevo Najman*, BIOLOGIJA SA HUMANOM GENETIKOM, Niš, Autori, 2001.

[3] *Jacon Price, Mike Gunderloy*, VISUAL C# .NET, Sybex, 2003.

[4] *Zixiang Xiong, Qiang Wu, and Kenneth R. Castlemen*, "Enhancement, Classification and Compression of Chromosome Images",

www.gensips.gatech.edu/proceedings/Contributed/CP2-03.pdf

[5] MetaSystem's karyotyping system Ikaros http://www.metasystems.de/products/ikaros/ikaros3.htm

[6] Spectral Imaging - BandView® EXPO System http://www.spectral-imaging.com/BandView.asp