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CCD microscopy - image analysis by Group for Intelligent Systems (GIS)

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BACKGROUND: *Classic microscopy does not allow dealing with visual and photographic phenomena, observation of the distinctions where they are needed, or carrying out of any image processing other than cutting. Digital imaging provided more detailed and distinctive insights, and formed the basis for the development of the image processing tools and techniques that transcended in potential and power all initial expectations. The purpose of this article is to present our achievements in this area.*

METHODS: *The starting point is that the matrix. Digital image is a discrete approximation of the continuous two-argument function. Analogue original is a fair basis to start building good mathematical representations of present objects, structures, features, which are then subjected to calculations, transformations and analyses that could precisely match the predefined aims. These analyses are: photomorphology 3D model with morphometrics and full 3D navigation, mathematical representations of granular forms in images, object contours, mathematical representations of chromosomes with mathematical definition of similarity, automatic procedures, such as pattern normalizations, matching, classifications, which leads to broader application of Artificial Intelligence methodology.*

RESULTS: *We developed a method and a complex software environment for microscopic imaging, with many tools and algorithms that proved to be useful in genetics, pathology, and oncology. The presented method is prepared and available for further generalizations and automatization, easily bridging to intelligent systems.*

CONCLUSION: *Microscopic imaging is powerful new high-tech domain of great assistance in biomedical research and medical practice that is revolutionizing real time diagnostic methods and potential, matching the power of molecular biology techniques. Being the pioneers in the microscopic imaging, we are pleased that it is exponentially expanding to the general benefit.*

KEY WORDS: *Chromosomes; Karyotyping; Microscopy, Electron, Scanning; Image interpretation, Computer-Assisted; Artificial Intelligence*

INTRODUCTION

CCD microscope is an integrated system that consists of the following components: a (high quality) microscope, CCD camera (with an adapter for focusing), computer (PC), and software (SW) for acquisition and analysis of microscopic images (Figures 1,2,3).

Figure 1. Microscope - CCD - PC - SW

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The system can be obtained as a complete set from all leading manufacturers of microscopes, or it can be realized by adding the so-called imaging system to any quality microscope.
The system enables the user to see better and more, to measure objects that were inaccessible up to now, to carry out new types of operations with the images that became possible by computer image processing. Improved archiving, search, comparison, as well as access via the Internet are easily provided by such systems. Such systems are widely introduced into medical practice, in spite of high prices, testifying that the results achieved in the field of this technology are necessary for medicine.

Figure 2. Camera - microscope connection

Figure 3. The image of a preparation and its analysis on the monitor

Along with high technology optical systems of the utmost significance here is high technology CCD element, and image processing and image analysis software. We were among the first in the world to begin development of these systems, and we are surely among the pioneers of CCD microscopy. Processing and analysis methods that form the basis of our software can be compared in every respect with the high quality world products. One of the drawbacks of usual commercial systems is that they are completely or partially closed systems (for the reasons of commercial safety), which prevents the user from expanding analytical methodology and exchange of results achieved with the help of this system on a wider scale.

SOFTWARE - PROGRAM APPLICATION AND APPLICABILITY

The already implemented algorithms are included into 5 program packages, 4 of which are completed and one is still in the process of development. They work under Win98, NT, WIN2K operating systems. Our software includes the following features/tools:
- All standard processing: contrast, light, rotations, sharpening, averaging, etc.;
- Five types of object selections: parallel to the frame of the picture, inclined parallelogram,
curved with the manual adjusting of curve geodesy, curved with rectification, area selection, spotted, which provide an adequate extraction of the necessary parts of the image;
- Photometric profiles of selected objects, similarity function of photometric representations for chromosomes and gels (Figure 4);

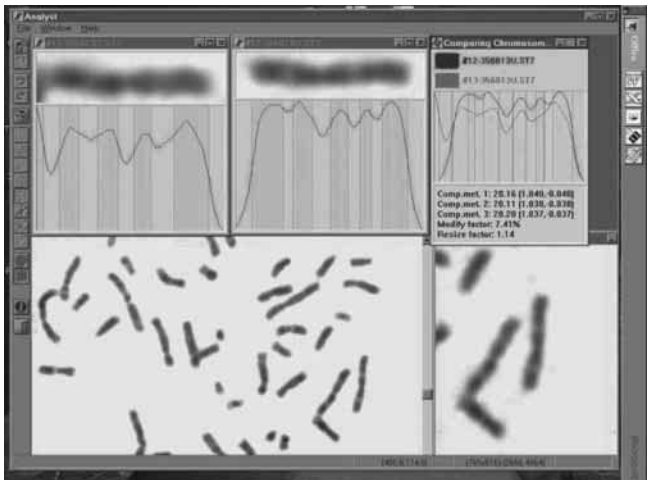

Figure 4. Trisomy confirmed by chromosome photometry

- 3D photomorphology, with 3D graphics, full 3D navigation, measurement and signal tools;
- Precise light measurement in defined image areas;
- Determining and counting granules of a chosen size;
- Surface morphometry;
- Synthesis of color composite images (monochromes obtained by acquisition in different wave lengths are combined using functional colors, with complete control of centering and weight relations);
- Automated separation of objects, normalization and rectification when necessary, sorting by length;
- Several kinds of image spectroscopy;
- The functional microscopic magnification improvement by two orders of magnitude on photomorphologic structures;
Our system is used to test weakly visible and invisible changes in chromosomes and nuclei (Figure 5), to determine genetic origin of pathological marker chromosomes (Figure 6-9), for quantification of in situ hybridized RNA in a neuron nucleus, precise positioning of gene signals, along with direct use of these procedures for similar applications of molecular biology techniques.

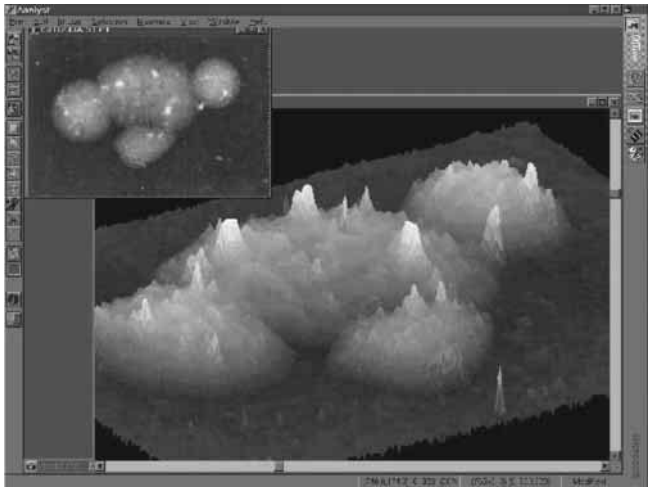

Figure 5. Trisomy confirmed by photomorphology of nuclei

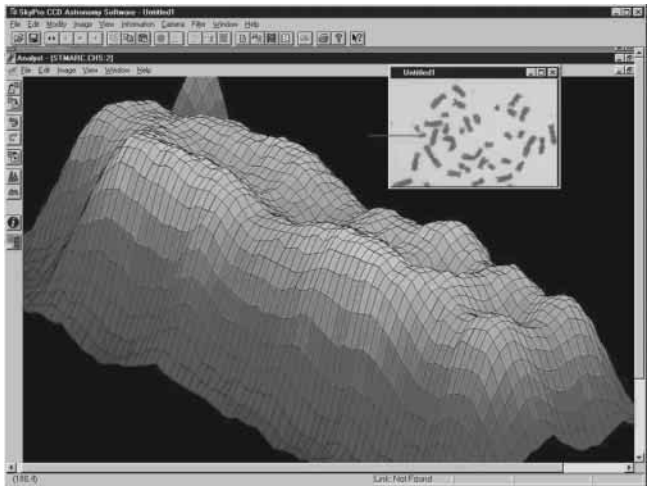

Figure 6. Defective marker chromosome photomorphology magnified over 100.000 times provides all structural details

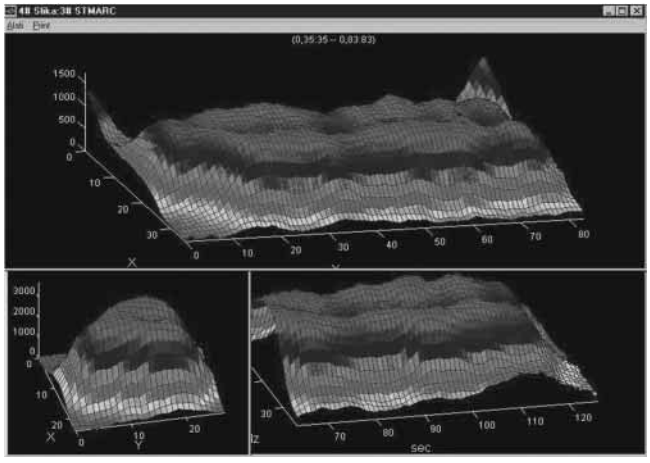

Figure 7. Mitosis with a defective chromosome pair - by precise 2-dimensional photomorphology in this example - rare hematology syndrome was identified after application of normalization algorithm on chromosomes: marker = Y + longer-arm-X

Figure 8. Mitosis with Philadelphia syndrome, elaborated below

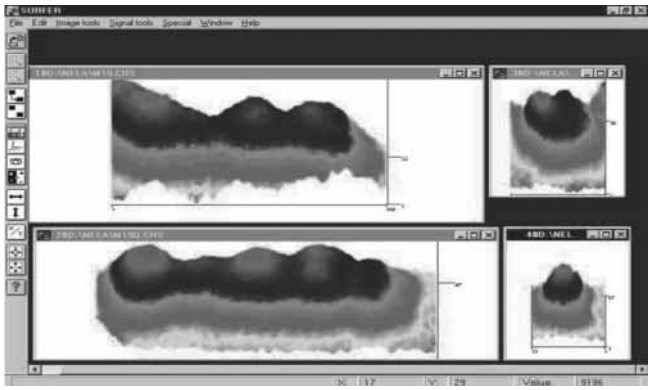

Figure 9. Photomorphologically confirmed Philadelphia syndrome. Above: normal pair; below: defect, transfer of a part of a chromosome from the shorter to the end of the longer one; all image processing done by our software for photomorphologic analysis

Thanks to the above-mentioned possibilities the software and CCD system that comprises it became very convenient for extensive applications in microscopy, especially in cytogenetics (testing changes in chromosomes and nuclei, detecting gene signals, in situ hybridization, fluorescent in situ hybridization), hematology, pathology, oncology, prenatal diagnostics. Of great significance are the additional possibilities of archiving, of monitoring the development and treatment of a disease, of a more objective comparison of analysis results, as well as of extended consultations - remote expertise via the Internet.

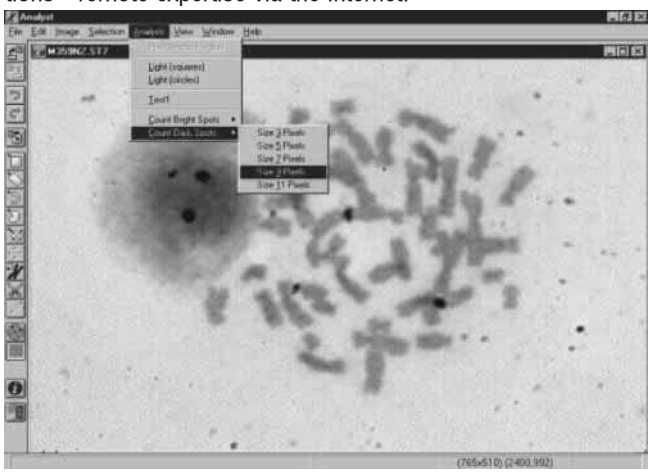

Figure 10a. Application of granular form detection: selection of size of granule

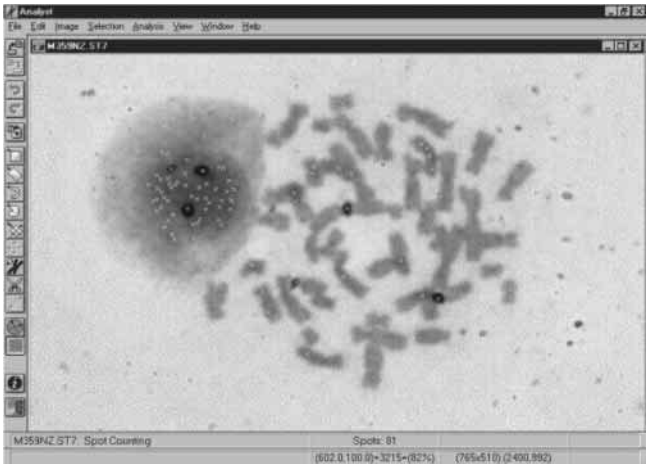

Figure 10b. Application of granular form detection: identified granule

Figures 10 to 12 show a potential of our software tools in work with in situ hybridized preparations, cell nuclei, chromosome signals and in neural cells nuclei.

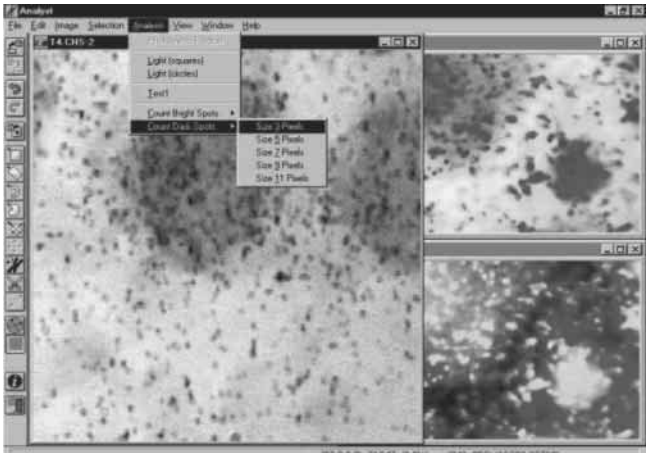

Figure 11. In situ hybridized RNA in a neuron nucleus

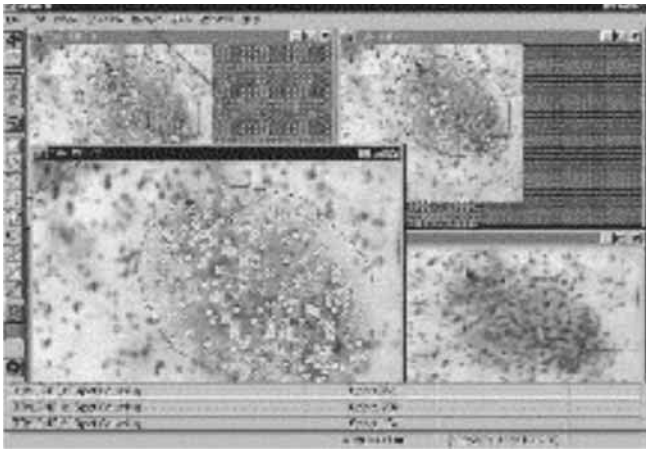

Figure 12. Automated determination and counting of granules

For the FISH (fluorescent in situ hybridization) techniques, we have developed and implemented a collection of useful tools that will facilitate precise addressing of signals on chromosomes, in spite of absence of standard banding patterns (Figures 13 and 14), noise reduction filters, monochrome image preprcessing and fusion into composite monochromes that are forwarded into free, user controlled color composites which could be very informative in a number of diverse uses, e.g. enhanced definition of investigated features, techniques with multiple signals and their precise relative distribution and relations. Input monochromes into color composite fusion, are preprocessed, then subjected to the fine centering, then the generation of a complete gallery of color composites is done in real time...Some examples of work with FISH preparations is illustrated in the sequence Figure 15 to Figure 19.

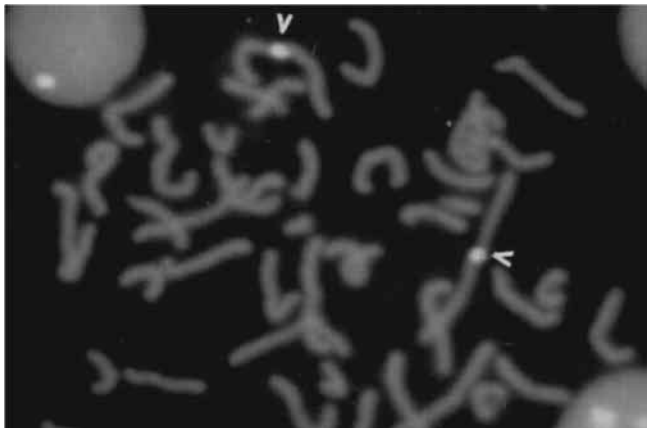

Figure 13. FISH signals on chromosomes

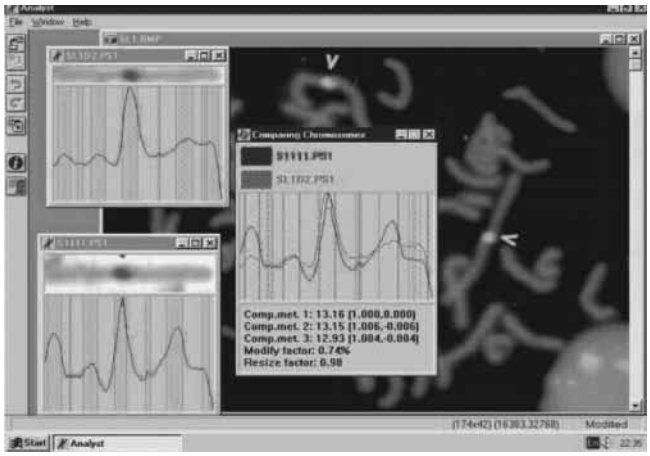

Figure 14. Precise positioning of a signal on a chromosome; photometric identification of a chromosome

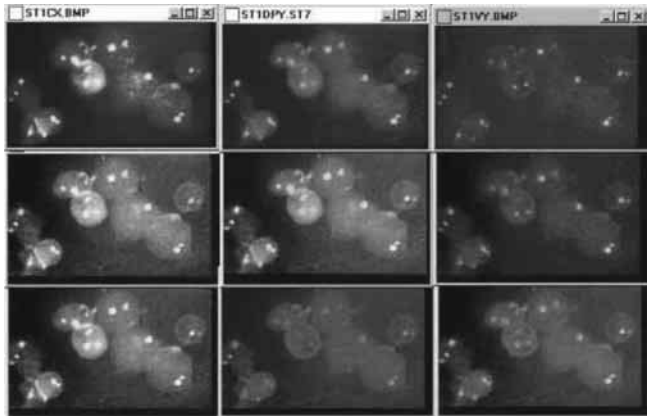

Figure 15. FISH - Colcomposites: color combining original monochromes from Figure 16; hidden structural features become visible e.g. trisomy in the central nucleus, well-defined nucleic contours; coloring is user controlled - in minutes

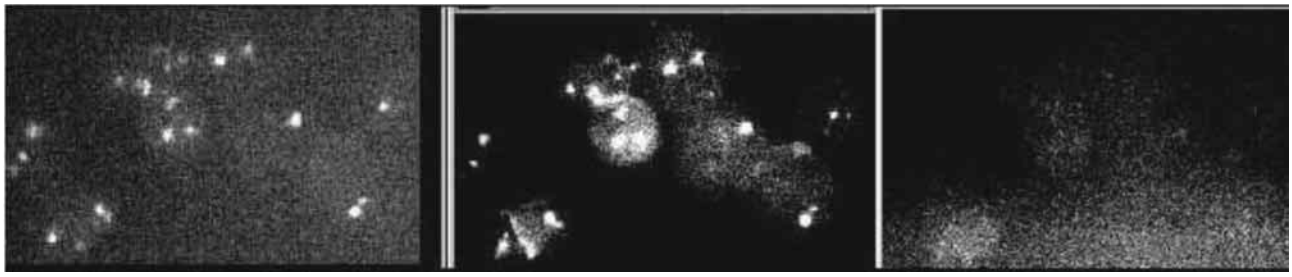

Figure 16. FISH photos of a group of nuclei in three wavelengths, image quality hardly usable

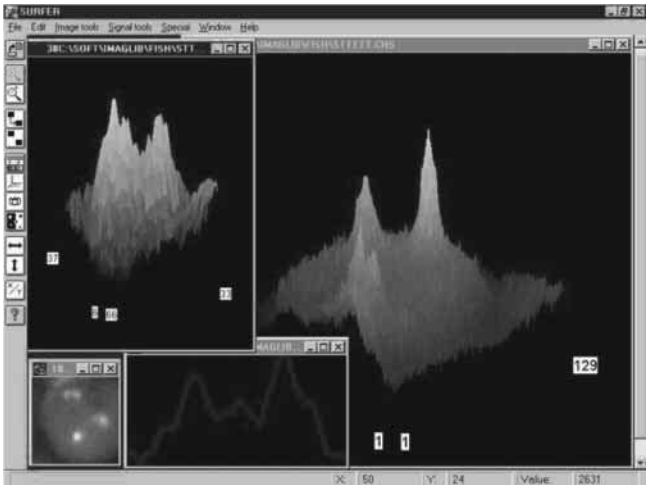

Figure 17. Photometric and photomorphologic measurements supported within full 3 dimensional navigation of intuitive and realistic visualization concept, resolved down to the image atom - pixel of high density astronomic quality CCD chips, provide unprecedented detail at high and highest magnifications

Automatic karyotyping, shown in Figure 20, after preliminary noise reduction, detects and extracts objects, constructing a curved coordinate system following the curved shape for each object, which is afterwards used for chromosome remapping as normalized, sorting them by length. User is allowed to "manually interfere" in the tracing of each chromosome coordinate system, define reconstruction parameters and stop "rectification" algorithm at a desired step. In this way, user will freeze chromosome rectification in critical step and save microstructures that would vanish in further "rectification". Here processed objects could be exported back to the morphology structure analysis that is shown earlier.

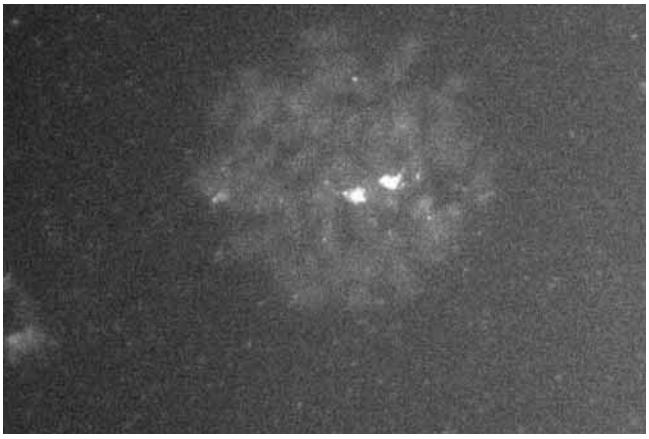

Figure 18a. FISH - mitosis one wavelength

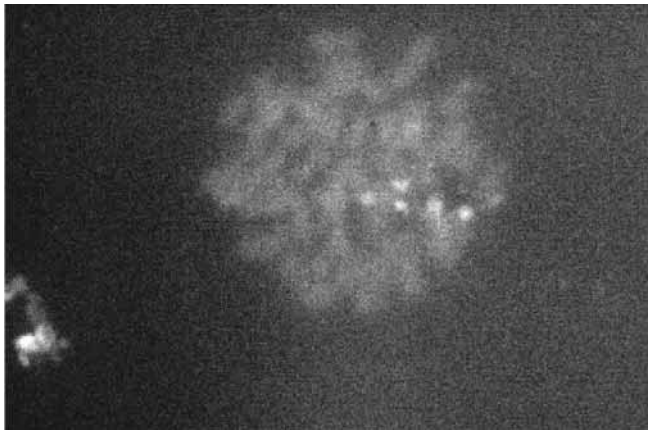

Figure 18b. ... second wavelength

Figure 18c. ... third wavelength

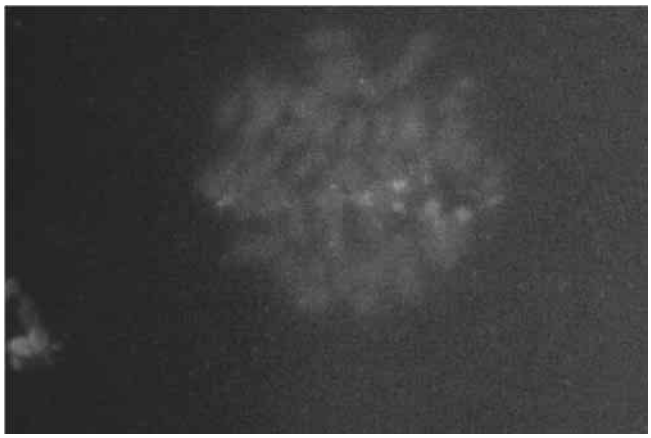

Figure 18d. ... and color composite, with removed some noise, better chromosome contour definition and signal color separation

Figure 19. User interface for color composite fusion, with precise recentering and color balance controls, shown with arrows, provide user with real time insight into color composite generation... (preprocessing and monochrome compositions not shown)

All our software also enables the user to connect easily to any other software and system. We plan continual evolution and support of all our software and systems. Its functions, which are useful for telemedicine sessions, are directly available using commercial service programs and we have good real time experience with our software.

We are capable of installing CCD imaging upgrade on every quality microscope, including older models, which is especially important, as world distributors of equipment deal with their models only.

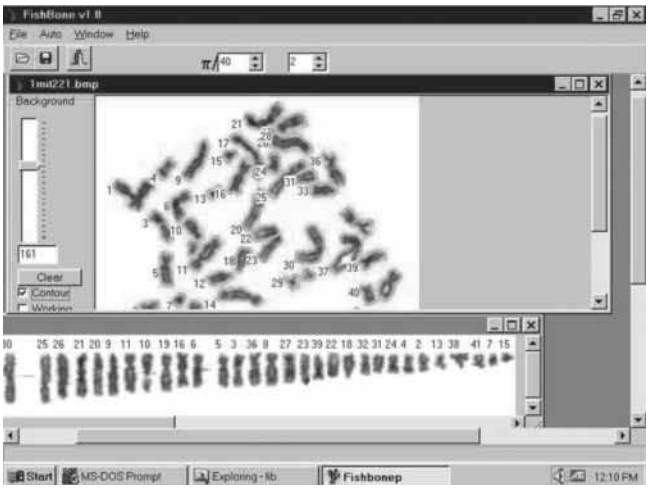

Figure 20. Automatic karyotyping

New CCD microscopes technology enables a faster, more precise, and more elaborate diagnosing, monitoring of patient condition and disease development, enables a faster and more precise determination of treatment methods. Total expenditure is reduced, and patients, especially those with the most serious diseases, have better chances.

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REFERENCES

1. Jovanovic A. Group for intelligent systems, problems and results, in Intelektualnie Systemi, tom 6. Moscow: Russian Academy of Sciences; 2002.
2. Jovanovic A. Matematika u biologiji. Belgrade: Matematicki fakultet; 1997.
3. Jovanovic A, editor. Image and signal processing (monograph on CD-ROM) GIS; 2002.
4. Group for Intelligent Systems, School of Mathematics, Un. of Belgrade (Intenet). Belgrade: School of Mathematics, © 1997-2003. Available from: http://www.gisss.com