

VIDEOTEST-KARYO 3.1

System for automatic karyotyping the human chromosomes with
an ability of training for automatic karyotyping the animal chromosomes
(with the software for Windows 98, 2000, XP)

developed by VideoTesT

SPECIFICATION



VideoTesT, Ltd.
190000 Russia, St.-Petersburg, P.O. Box 356
Phone: +7(812)314-8100, 314-8445
Fax: +7(812)325-6494
e-mail: info@videotest.ru
<http://www.videotest.ru>

VideoTesT-Metal © 1999-2004 VideoTesT, Ltd. All rights reserved.

Information in this document is subject to change without notice.

This document is a property of VideoTesT, Ltd. It may be copied or transmitted to a third party only in its complete form with no omissions; all trade marks and copyrights be preserved. Any changes or amendments shall be agreed upon in writing with VideoTesT, Ltd.

1. INTRODUCTION

VideoTesT-Karyo system is designed for automatic karyotyping of the human chromosomes with high accuracy and for analysis of the human chromosomes including an ability to create the chromosome ideograms. The system can work with the images of G-, Q-, and R- banded chromosomes.

With this system it is also possible to automatically karyotype the pig chromosomes. The system is trainable, and it is possible to train it to automatically karyotype the chromosomes of other animals. The manual mode is used to arrange the plant chromosomes into the karyogram.

VIDEOTEST-KARYO SYSTEM COMPONENTS

VideoTesT-Karyo automatic karyotyping system (see Figure 1) includes the following parts:

1. Upright transmitted light (or fluorescence) microscope,
2. Monochrome digital image acquisition system to capture the chromosome images from microscope to PC,
3. Computer and printer,
4. VideoTesT-Karyo 3.1 specialized software for automatic karyotyping and creating chromosome ideograms.



Figure 1. VideoTesT-Karyo system

The microscope is an important part of the complete system. It shall provide the sharp and contrast images of the chromosomes. The microscope used for VideoTesT-Karyo system shall comply with the following requirements: flat and evenly illuminated image field, Koeler illumination properly adjusted, high resolution, high image contrast, stabilized power supply unit, conversion filter included. The stage micrometer shall be included for system calibration.

The microscope shall be equipped with a 1x video adapter for installation of the image acquisition system, 10x (or 20x) lens to search for the good metaphase spread images for analysis, and 100x lens to capture the selected images for further analysis.

The image acquisition system shall capture the high quality images from the microscope to the computer. The image acquisition system recommended for capturing the images and their further analysis in the software: high performance monochrome digital camera with the high resolution (1280*1024 or more recommended), signal to noise ratio of 56 db or more, dynamic diapason of 8 bit or more. The camera driver shall provide the shutter automatic control. The camera control (the settings adjustment and image capture) shall be performed directly from the VideoTesT-Karyo software for comfortable and fast operation.

The computer and its operation system shall provide fast and reliable system operation and processing the big volumes of information. The recommended PC configuration for VideoTesT-Karyo system is as follows:

- Pentium 4 1,6 GHz or higher processor,
- 32 Mb videocard or higher,

- 512 Mb RAM,
- Windows XP Professional operation system,
- 17" monitor with 1280x1024 or higher resolution,
- 150Mb free hard disk space (hard disk Fast HDD, ATA100 or SCSI),
- Windows compatible mouse or other pointing device,
- Keyboard and CD-ROM,
- Free USB port.

GENERAL REQUIREMENT TO THE CHROMOSOME SAMPLES

Requirements to the metaphase spreads. The metaphase spreads shall be prepared in accordance with the standard techniques. The chromosomes shall be contrast, have sharp contours and visible bands. The software can process G, Q, and R banded chromosomes. The metaphase spread selected for the automatic analysis shall contain the chromosomes which lie not too close to each other, the number of contacting and overlapping chromosomes shall be minimum. On the other hand the chromosome scattering shall not be very big. The image background shall be clean and uniform. The poor image quality increases the total time of analysis and decreases the accuracy of automatic karyotyping.

The example images of the quality metaphase spreads are given on Figure 2.

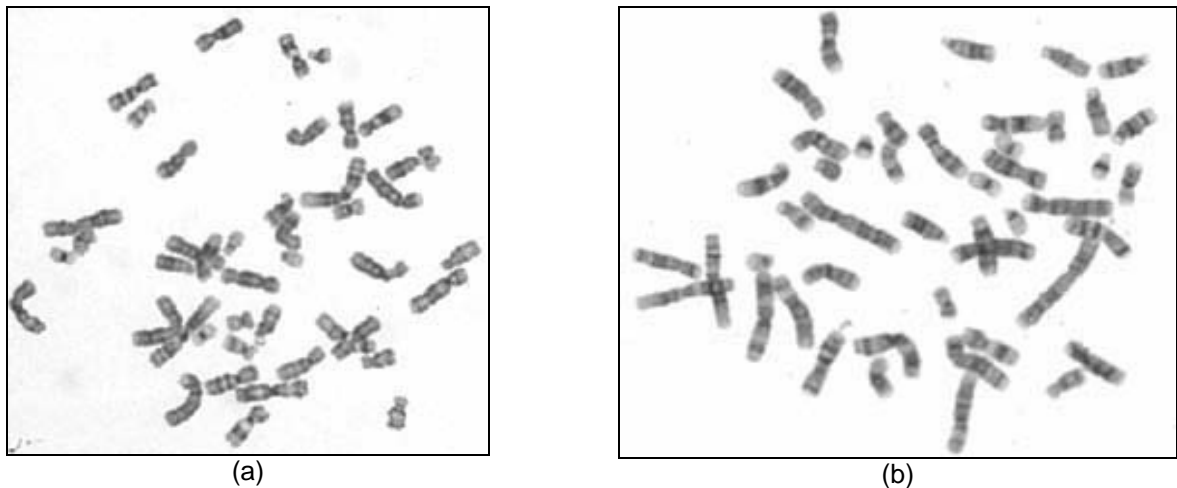


Figure 2. Example images of the sperm smears

2. VIDEOTEST-KARYO SYSTEM OPERATION

The metaphase spread processing in VideoTesT-Karyo is performed by the following steps:

1. Acquisition of the metaphase spread image and its preparation for analysis,
2. Automatic chromosome thresholding and work with the thresholded chromosomes
3. Arrangement chromosomes in a karyogram and work with the karyogram,
4. Chromosome comparison and creating the ideograms,
5. Save and printing out the analysis results,
6. Archive images and data in the built-in image database.

ACQUISITION OF THE METAPHASE SPREAD IMAGE AND ITS PREPARATION FOR ANALYSIS

VideoTesT-Karyo system provides an ability to use the following methods of image acquisition:

- Capture images with a digital image acquisition systems installed on a microscope,
- Open images from files (tif, bmp, and jpg file formats are supported),
- Copy images from the clipboard.

When the chromosome images are acquired with an image acquisition system their reduced copies are displayed in the special image bar, and the user can select a desired image from this bar for analysis (see Figure 3).

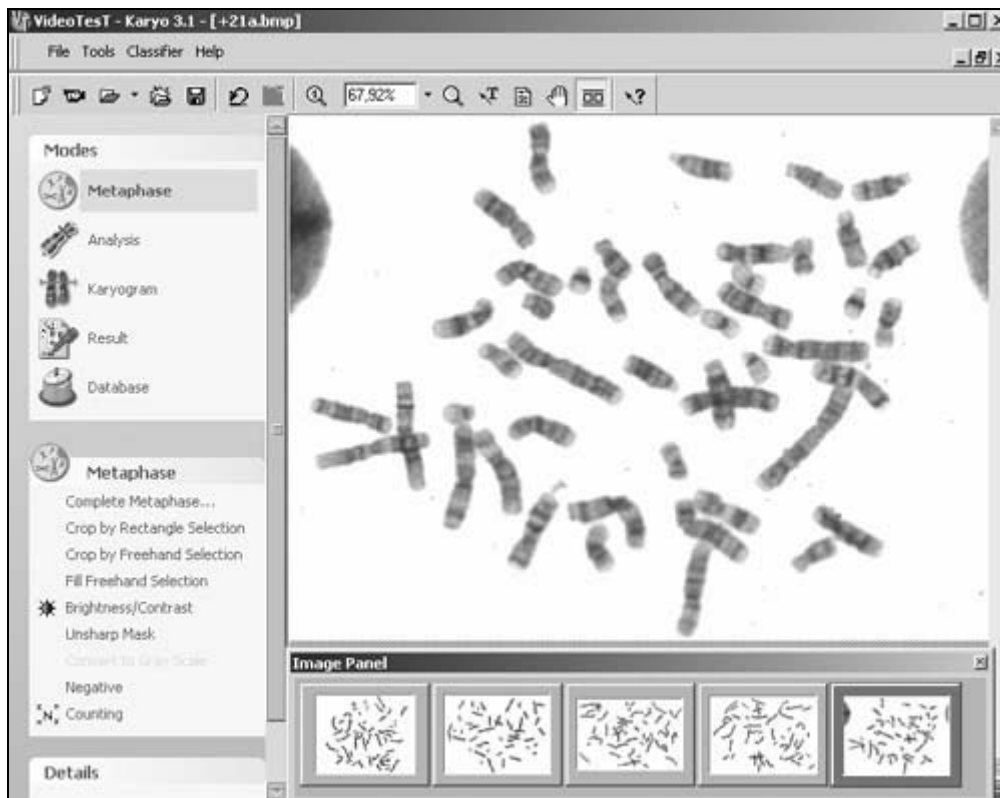


Figure 3. Image bar (in the bottom).
The active image is displayed in the software window

After the image is selected the user can prepare it for the automatic analysis. The following abilities are provided:

1. Add the metaphase spread. This operation is used in case if the complete metaphase does not go in a frame, and there are chromosomes which are out of the frame. If it is so you can move the microscope stage, acquire the next image, which contains the missing chromosomes, and move them to the first image.
2. Ability to outline and copy the rectangle or free image area and paste it as a new image (see Figure 4). This is necessary in case if chromosomes are located in the image center, and if there are different artifacts on the source image.

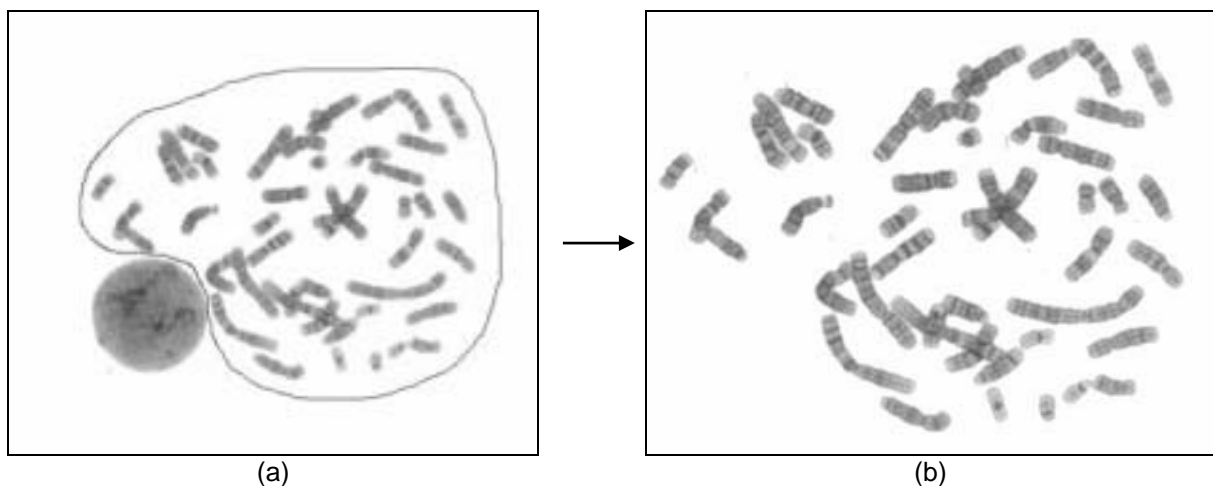
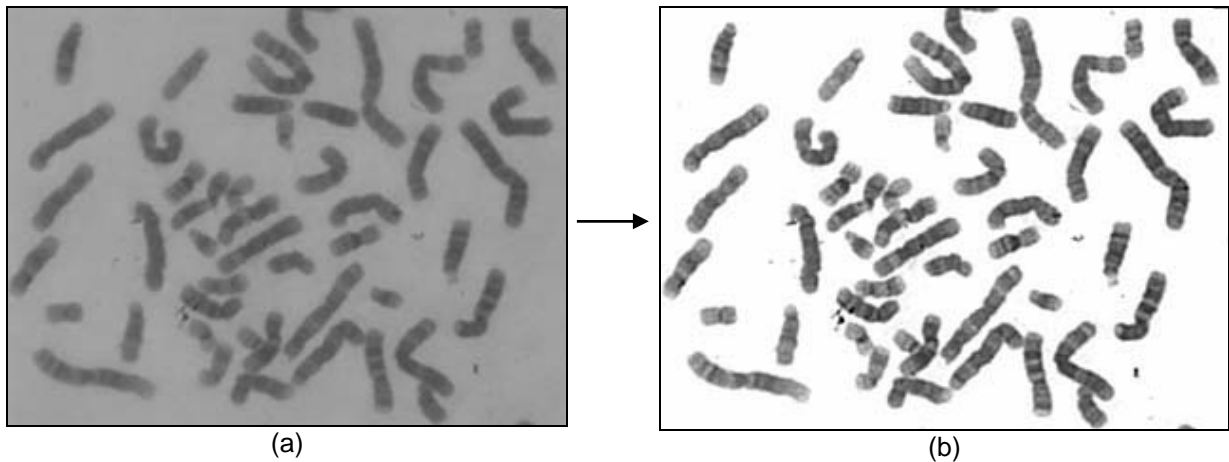


Figure 4. copy and paste the image area

3. Filling the image area. This operation is required in case if it is necessary to remove the image area or image object (s).
4. Image enhancement: brightness, contrast, and sharpness improvement (see Figure 5). Necessity of image enhancement is minimum if all requirements to the metaphase spread preparation, microscope and image acquisition system adjustment are met.



(a) (b)
Figure 5. Source image enhancement. a – source image,
b – enhanced image

On the image opened in the software window it is possible to perform the manual chromosome counting.

The annotations (graphics and comments) can be added to the metaphase spread image.

AUTOMATIC CHROMOSOME THRESHOLDING AND WORK WITH THRESHOLDED CHROMOSOMES

The chromosome thresholding on the image is performed automatically. The user can adjust the chromosome thresholding if necessary. The chromosomes are thresholded by difference in brightness between chromosomes and image background. If the difference is small the chromosomes will be thresholded poorly.

After the chromosomes are thresholded, the touching and overlapping chromosomes are separated automatically, and the central lines and centromeres are drawn (see Figure 6).

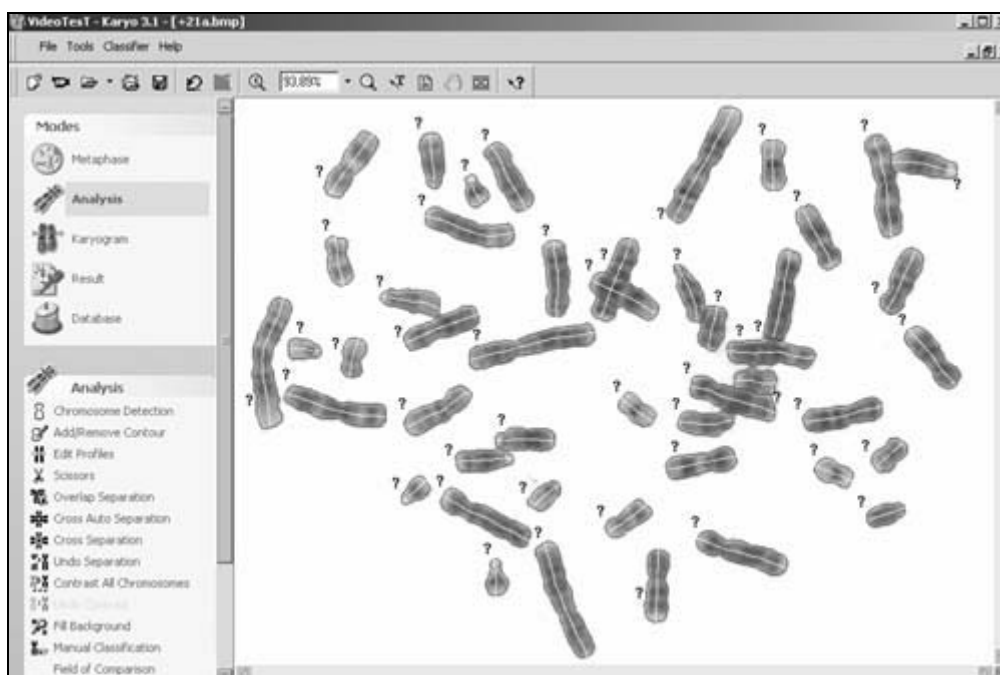


Figure 6. Automatic chromosome thresholding

The user can control the automatic thresholding results and make corrections if necessary. The following correction abilities are included:

1. Correct the chromosome contour. It is necessary when some chromosome parts failed to be thresholded because their brightness was close to the background.
2. Separate touching chromosomes, separate overlapping and crossing chromosomes (see Figure 7). This operation is required if some touching or overlapping chromosomes have been not separated automatically.

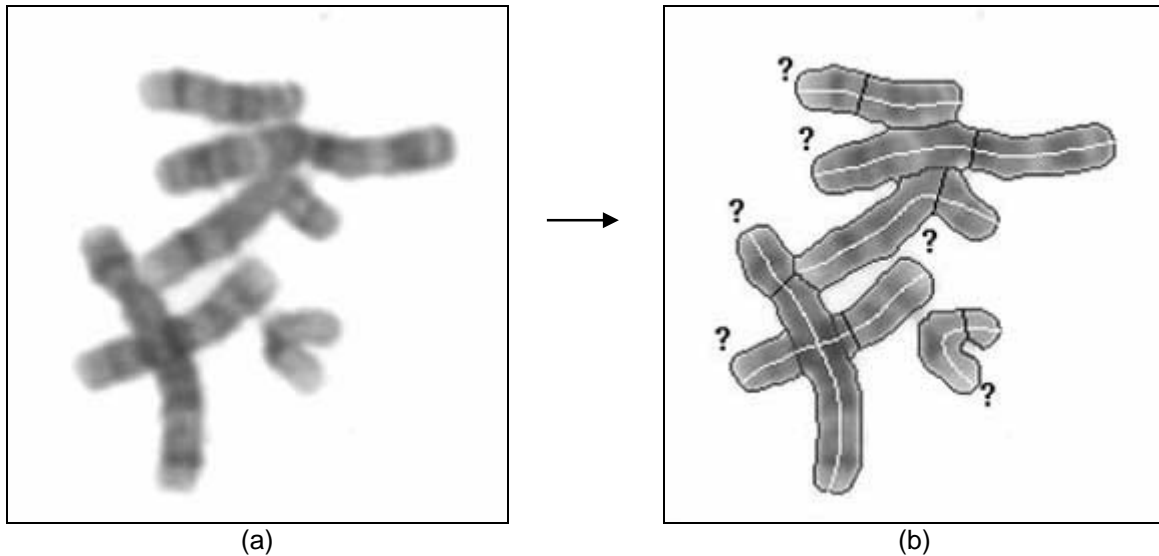


Figure 7. Separation of the touching and overlapping chromosomes

3. Undo separation. This operation is required if the excessive separation within a chromosome has been performed automatically.
4. Correction of the central line and centromer position if necessary (see Figure 8). Inaccuracies in central lines and centromers positions just slightly influence on the accuracy of automatic karyotyping, however they influence on the chromosome position in the karyogram (chromosome alignment) and on the performance of the "Straighten chromosome" operation.

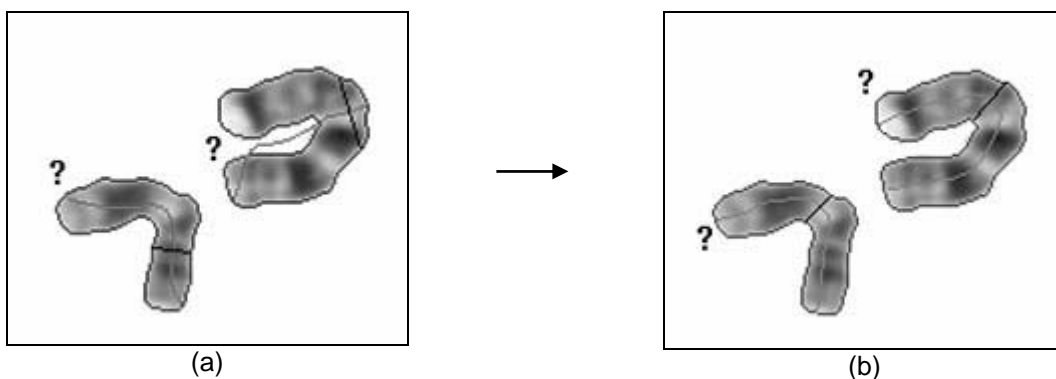


Figure 8. Correction of the central line and centromer position

In this mode it is also possible to contrast all chromosomes and fill the background in with the selected color.

It is also possible to manually assign numbers to the chromosomes, and then they will be arranged in the karyogram in accordance with the assigned numbers.

KARYOGRAM

After all thresholded chromosomes are corrected (the necessity of corrections is minimized if the chromosome images are of good quality), they will be arranged in the karyogram (see Figure 9).

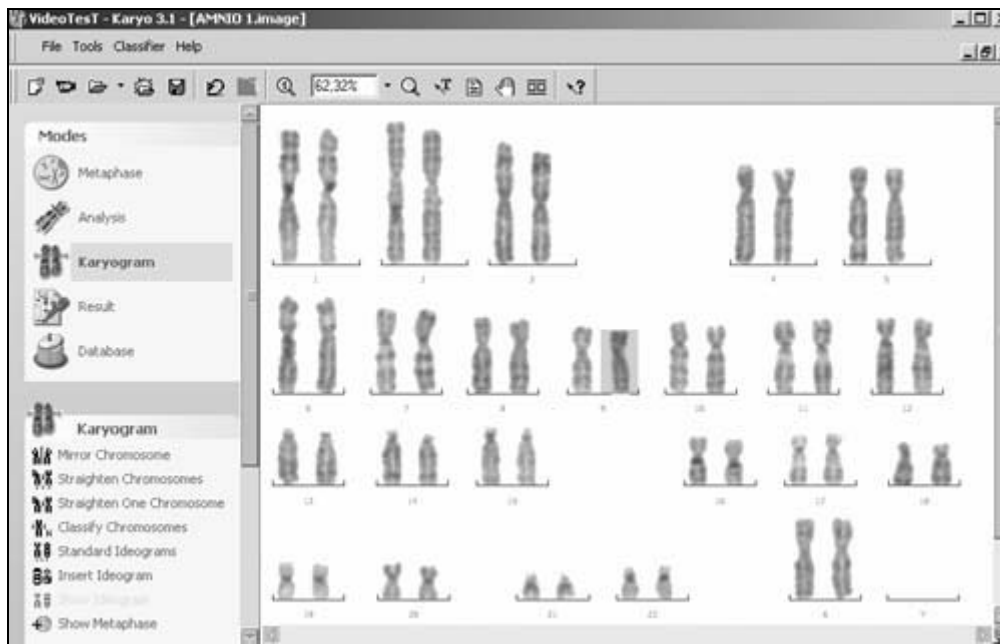


Figure 9. Karyogram in the software window. The chromosomes are straightened.

In the karyogram all chromosomes are aligned by their centromeres, bottoms or upper tips. The karyogram background and some other settings are adjusted by the user.

In the karyogram it is possible to change the chromosome positions by drag-and-drop operation, move them up and down against the alignment line, rotate by 180 or by the free angle, mirror and straighten (all or selected chromosomes). If necessary it is possible to display the source metaphase spread together with the karyogram in the software window.

Together with the karyogram it is possible to display the source metaphase image if necessary (see Figure 10).

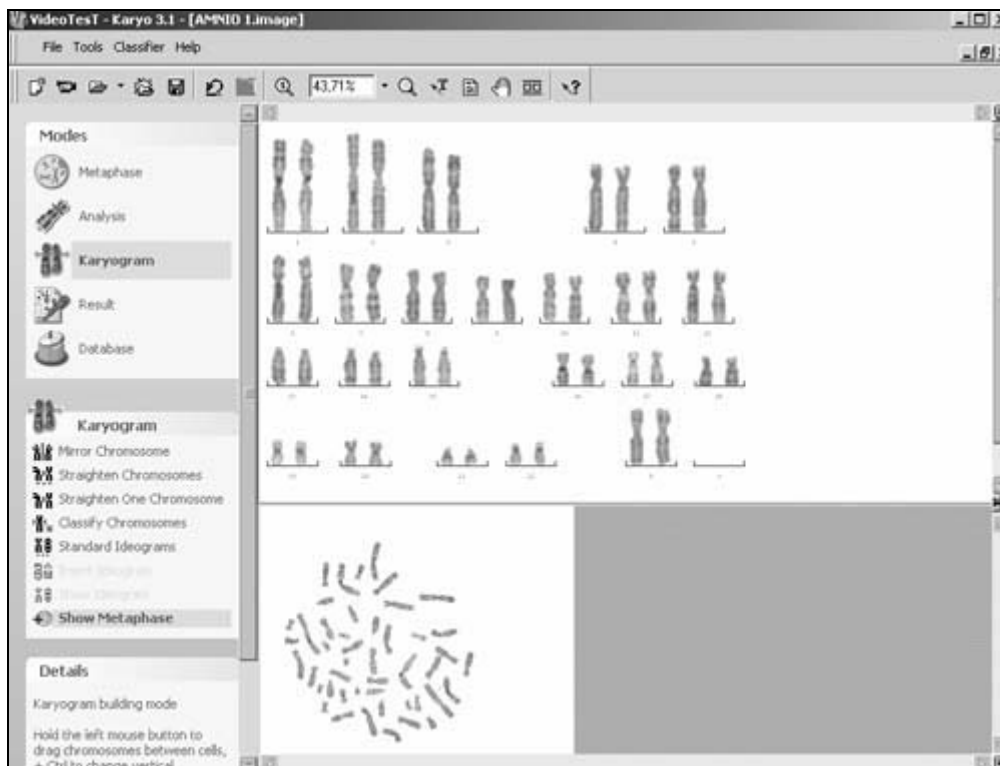


Figure 10. Karyogram and metaphase in the software window

In the karyogram it is possible to display the standard chromosome ideograms (with 400, 550 or 850 resolution depending on the selected standard ideogram database). The standard ideograms can be displayed either for all or for the selected chromosomes (see Figure 11).

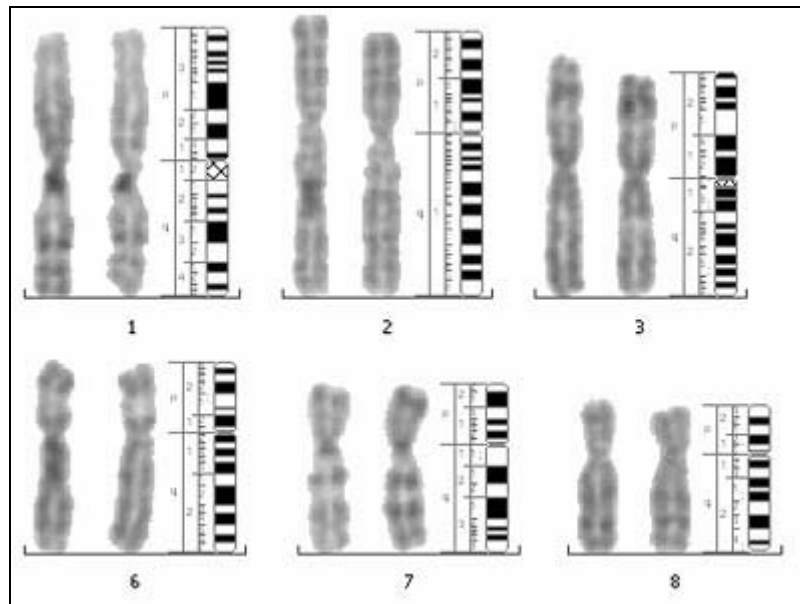


Figure 11. Standard ideograms in the karyogram.
Karyogram fragment

VideoTesT-Karyo 3.1 software provides an ability to type the text comments on the karyogram image (see Figure 12).

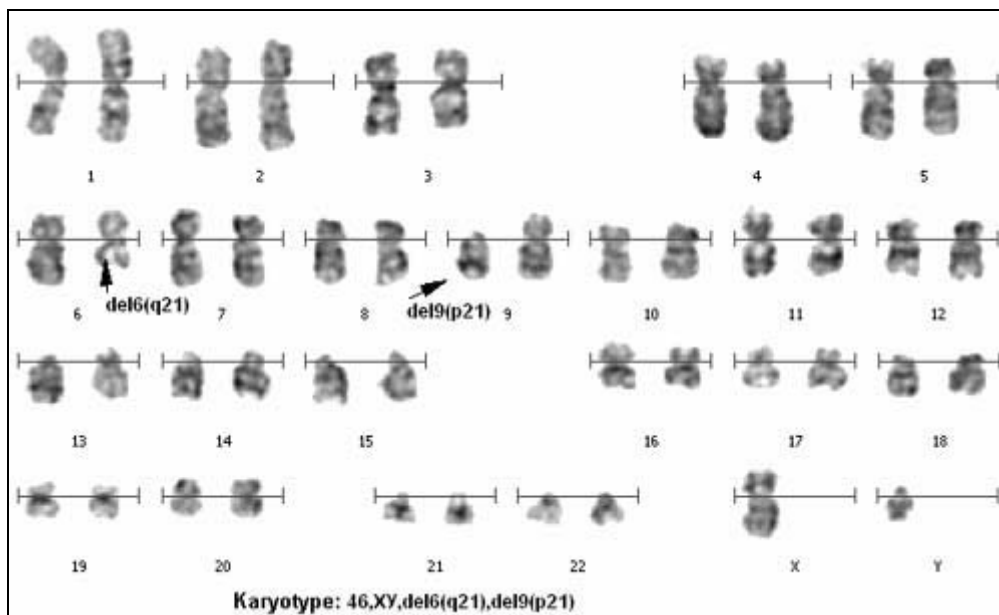


Figure 12. Karyogram with comments

CHROMOSOME COMPARISON AND IDEOGRAMS

Comparison of the chromosomes between each other and with ideograms is performed in the field of comparison where they are dragged from the image.

In the field of comparison the chromosomes can be straightened and scaled (see Figure 13). The standard ideograms from the ideogram database can be displayed in the field of comparison together with the corresponding chromosomes.

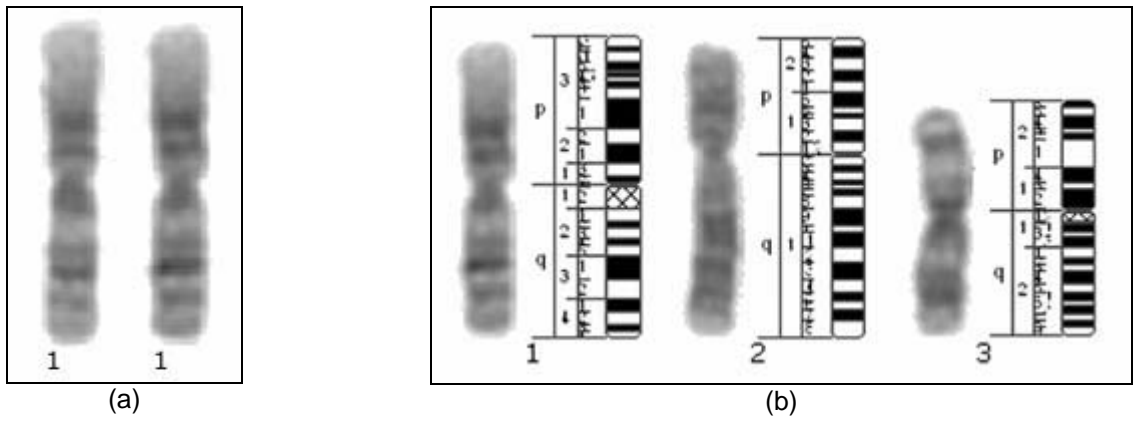


Figure 13. Field of comparison. a – straightened and scaled chromosomes, b - chromosomes with standard ideograms

When a chromosome is dragged to the comparison field the ideogram template will be displayed next to it. The user can create the chromosome ideogram: draw the band boundaries, shade the bands, and assign the numbers (see Figure 14). The created chromosome ideogram can be sent to the ideogram database.

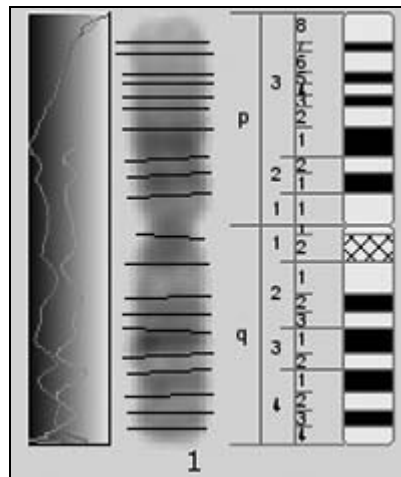


Figure 14. The ideogram creation in the comparison field

When the analysis is finished the user enters patient data into the Patient Data form (see Figure 15). If necessary the form view and fields can be modified.

The screenshot shows a 'Patient Data' dialog box with the following fields and values:

- Patient: Liza Perkin
- Sex: female
- Age: 3
- Material of analysis: peripheral blood
- Clinical diagnosis: Down Syndrome
- Karyotype: 47,XX,+21
- Medical comment: Down Syndrome
- Analyst: Susane Dreem
- Date of analysis: 19.08.2004

Buttons at the bottom: OK, Cancel, Default List, Help.

Figure 15. The patient data form

SAVE AND PRINT OUT THE ANALYSIS RESULTS

The chromosome images can be saved to a file with .tif, .bmp, or .jpg extension. If necessary the original image can be saved together with the karyogram to a file with .image extension. It is also possible to save the active document view to a .jpg file, thus providing an ability to open the karyogram in any standard application working with .jpg file format.

The analysis results can be printed out (see Figure 16). The VideoTesT-Karyo 3.1 software includes a number of pre-installed printing templates. If necessary it is possible to modify the existing printing templates and create new templates. The report to be printed out contains data entered into the patient data form.

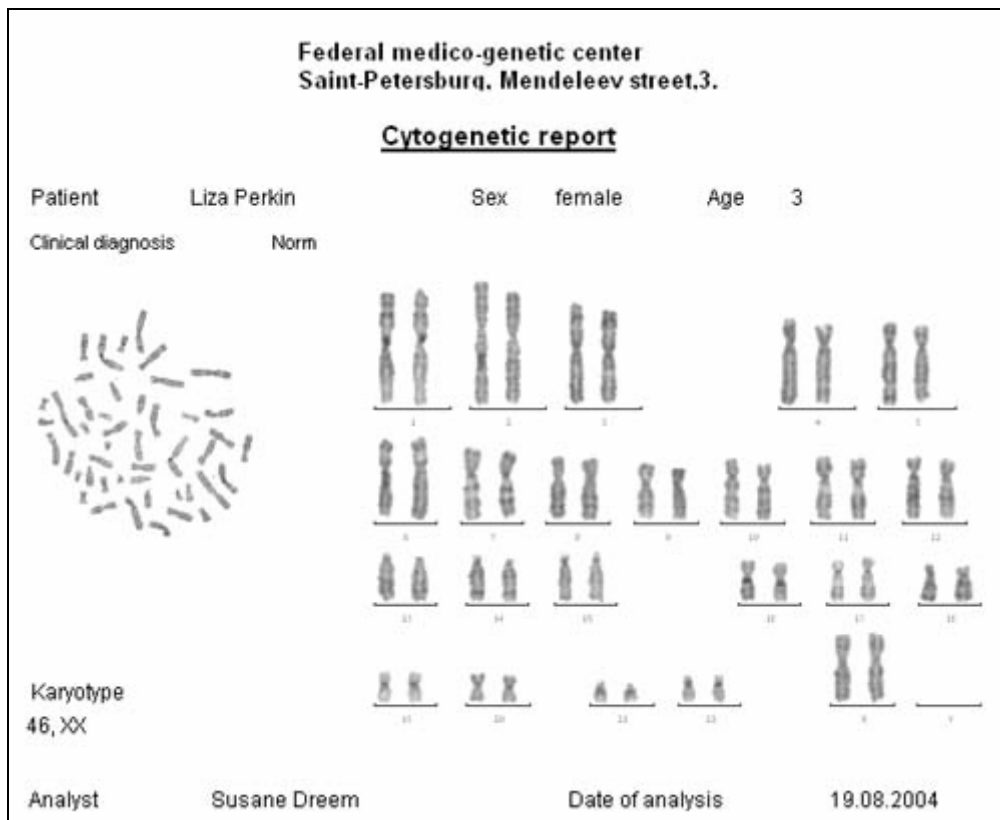


Figure 16. Standard report view

SAVE IMAGES AND ANALYSIS RESULTS IN THE IMAGE DATABASE

VideoTesT-Karyo 3.1 software includes the integrated image database.

The image database is designed for archiving, sort and quick search for images, karyograms, and supporting information (patient data, text comments).

The image database has the following abilities:

- Store images in compressed form with no loss in quality,
- Support images with necessary information,
- Multi-level sort and quick search for the desired information by key words, text, dates, numbers, etc., data sampling,
- Ability to create and manage several databases,
- Ability to modify the blank view, database fields number and types.

When the user opens the image database in the software window, the database blank is displayed (see Figure 17).

The screenshot shows a data entry form for a karyotype analysis. The fields are as follows:

- Patient: Anna Wiley
- Code: 2341
- Age: 5
- Sex: Female
- Material: Peripheral blood
- Staining: G-banding
- Clinical diagnosis: (empty)
- Karyotype: 47,XX,+13
- Date of sampling: 02.07.2002
- Date of analysis: 04.07.2002
- Analyst: Dr. Williams
- Operator: (empty)

At the bottom, there are two image thumbnails: "Metaphase plate" showing a cluster of chromosomes and "Karyogram" showing a structured arrangement of chromosomes.

Figure 17. One of possible image database views (Blank)

The image database can be displayed in three modes: **Blank**, **Gallery**, and **Table**.

In the **Blank** mode (see Figure 17) the full database record is displayed on the screen. The record contains a number of fields (set by the user) arranged as the user defined. In the Blank mode it is possible to enter the necessary information into the database.

The Blank can contain the following fields: chromosome image, karyogram, text, number, and date fields. The fields are labeled and filled in by the user. Some fields are filled in automatically. The patient data entered in the Patient Data form is sent there (see Figure 14). The text fields can contain the unlimited number of symbols. By default in all date fields the current date is displayed.

In the **Gallery** mode the thumbnails of all database images are displayed (see Figure 18). The database field to be displayed in the Gallery, and its size are set by the user.

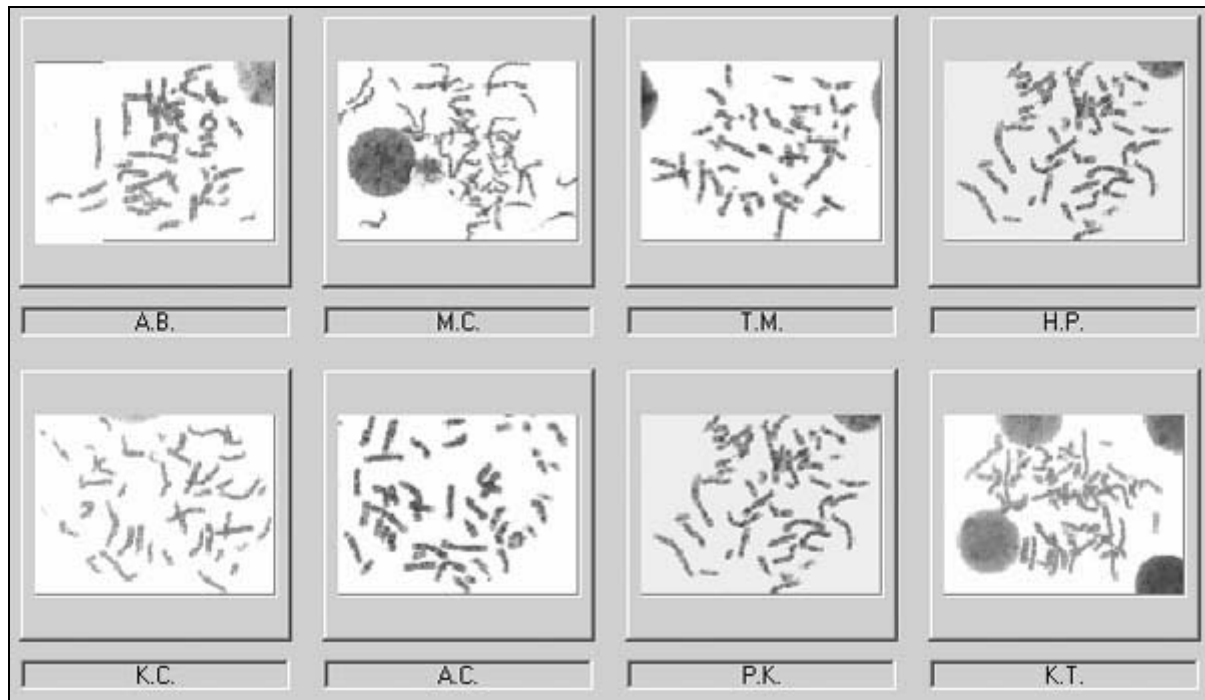


Figure 18. Image database in Gallery mode

In the **Table** mode the database records are displayed in the table form (see Figure 19). The table mode is used for entries sort and filtration. The image and karyogram fields are not displayed in the table.

Patient	Sex	Clinical diagnosis	Material	Staining	Date of analysis	Karyotype
Anna Wiley	Female	?	Peripheral blood	G-banding	04.07.2002	47,XX+13
Eva Miler	Female	Turner's Syndrome	Peripheral blood	G-banding	06.07.2002	del(X)(p10)
Liza Perkin	female	Down Syndrome			19.08.2004	47, XX, +21
Maria Smith	Female	Norm	Chorion	G-banding	03.07.2002	46,XX
Mike Jackson	Male	Norm	Peripheral blood	Q-banding	05.07.2002	46,XY
Piter Cleits	Male	Down Syndrome			19.08.2004	47, XY, +21

Figure 19. Image database in the table mode

The chromosome images from the database can be sent to the karyotyping software for analysis, and the images and karyograms can be saved automatically in the image database if necessary.

The image database provides an ability to filter entries by any condition(s), e.g. by the analysis date, type of material, patient name, etc. The database entries can be sorted by any field (text, number, or date fields). The image database provides an ability to search for the text fragments in the text, number, or date fields.

The user can create and manage several databases, each having its own fields and layout.

3. ADDITIONAL ABILITIES

VideoTesT-Karyo 3.1 system performs the automatic karyotyping the human chromosomes with high accuracy (up to 95%). The software also provides an ability to automatically karyotype the pig chromosomes.

VideoTesT-Karyo 3.1 is a trainable system. It means that the user can train the software to automatically recognize and karyotype the human chromosomes on his own smears. It is also possible to train the system to automatically recognize the animal chromosomes. In order to train VideoTesT-Karyo 3.1 to automatically recognize the animal chromosomes it is necessary to perform the following operations:

1. Create the karyogram template,
2. Acquire at least 40-50 good quality chromosome spreads of the studied animal,
3. Threshold chromosomes on all images and manually assign their numbers,
4. Send images with the recognized chromosomes to the classifier, and thus train it to automatically recognize the animal chromosomes.

Then it is possible to use VideoTesT-Karyo 3.1 system for automatic karyotyping the animal chromosomes.

VideoTesT-Karyo 3.1 includes the following standard ideogram databases:

- human with 400, 550, and 850 resolution,
- mouse,
- rat,
- cattle,
- sheep,
- pig,
- hen.

By the user request VideoTesT-Karyo 3.1 software can be added with the standard ideogram databases of the other animals and plants.

4. ABOUT DEVELOPER

Since 1990 VideoTesT, Ltd. has been focusing its activity on development of image analysis software, integration of PC-based VideoTesT Image Analysis Systems, automation and implementation of the image analysis methods in Life Sciences, Medicine, Materials Sciences, and Manufacturing fields.

VideoTesT software includes several universal and specialized programs.

Universal programs:

- *VideoTesT-Size 5.0* – new software for image acquisition, annotation, enhancement and manual measurements on the images
- *VideoTesT-Album 4.0* - image database.

Special programs:

- *VideoTesT-Master (Morphology) 4.0* – image analysis in life sciences with pre-installed bio-medical routines
- *VideoTesT-Master (Structure) 4.0* - image analysis in material sciences with pre-installed material analysis routines
- *VideoTesT-Metal 1.0* – metal structure analysis
- *VideoTesT-Karyo 3.0* - chromosome analysis: karyotyping and ideograms
- *VideoTesT-Karyo 3.1* – high accuracy of automatic karyotyping the human and pig chromosomes, ability to train the software to automatically recognize the animal chromosomes
- *VideoTesT-FISH* - FISH analysis
- *VideoTesT-CGH* - CGH analysis
- *VideoTesT-Sperm 2.1* - sperm analysis (motility, concentration, and morphology). Ability to adjust the software for animal sperm analysis
- *VideoTesT-Colony Count* - count and sizing the bacteria colonies.

For additional information on VideoTesT products, please contact:

VideoTesT, Ltd.,
190000 Russia, St.-Petersburg, P.O. Box 356
Phone: +7(812)314-8100, +7(812)314-8445
Fax: +7(812)325-6494
e-mail: info@videotest.ru
<http://www.videotest.ru>