

INTERACTIVE SYSTEM FOR AUTOMATIC ANALYSIS OF HUMAN R-BANDED METAPHASES

1. Interactive Procedure in Selection of Metaphase Digitized Images

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A computer system analysing human metaphase images and finding banding patterns of R-banded chromosomes has been set up. This first paper describes the interactive procedure in selection of metaphase images, automatically digitized starting from microphotographic negatives.

Systems for automatic analysis of human metaphases prepared according to conventional methods have been developed in several laboratories (Ledley 1964 and 1969, Rutovitz 1968, Gallus 1968, Gallus et al. 1969, Mendelsohn et al. 1969, Gallus and Neurath 1970, Neurath et al. 1970, Rutovitz et al. 1970, Gallus and Regoliosi 1973).

The results are promising, but not completely satisfactory since such chromosome spreads do not contain sufficient information to identify all the chromosomes of the human karyotype (Lubs and Ledley 1973).

New banding techniques provide a further class of information for chromosome analysis (Dutrillaux 1974). Accordingly, some laboratories have reexamined the possibility of setting out programs for human metaphase automatic analysis. Some results have already been described (Lubs and Ledley 1973, Castleman and Wall 1973).

Recently, we have set up a program for human R-banded metaphases automatic analysis (Forabosco et al. 1975). Our program has been now included in an interactive system by means of which we aim to obtain automatic karyotype diagnosis (Fig. 1).

There is now general agreement that an automatic human karyotype analysis can only be obtained in an interactive system. Our system needs the interference of an operator in two phases of the analysis: (1) selection of metaphases to be analysed; (2) karyogram evaluation.

The selection of metaphases is a fundamental stage for obtaining correct results from the whole system. This step requires an operator's intervention to decide which metaphases must be analysed and to correct acquired data in order to utilize metaphases otherwise not analysable.

The type of operator's intervention is related with the type of program to be employed. However, some interventions are always necessary whatever the procedure may be.

The present paper describes an interactive procedure set up for the selection of metaphases digitized starting from microphotographic negatives. This procedure overcomes some troubles normally present in the metaphases employed for automatic analysis, and can be also utilized for automatic analysis of metaphases based on programs different from ours.

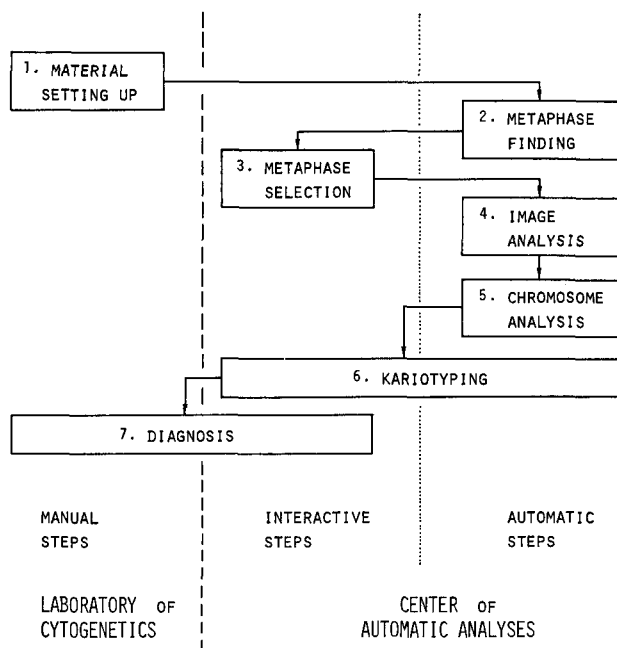


Fig. 1. Scheme representing steps for automatic diagnosis of human karyotype.

COMPONENTS OF THE SYSTEM

Hardware

The present equipment is shown in Figs. 2 and 3. It consists of an IBM 360/44 computer, with a core memory size of 32 K words, a computer-controlled flying spot scanner (Proteus), and an interactive communication system (CNAF, Bologna, Italy). It has been recently described in detail elsewhere (Matteuzzi et al. 1975). Briefly, 35 mm negatives are digitized by Proteus to 64 grey levels. This is performed at a resolution of 512×672 points per frame, while the chosen points are 400×480 (See Technical Notes, 1.) Film advance, that is, the replacement of the examined frame, is under program control. The system can automatically manipulate spot size, brightness range, scanning density and coordinates of the scanning window. The scanned picture can be displayed on a small monitor screen during data transmission into computer's memory. After the spread is stored as a numerical array of grey levels, this digital picture is displayed on a large monitor screen which permits the discrimination of 6 grey levels and an enlargement of any section of the picture for more detailed study. A total of 14 switches and a bright spot or a light pen enable rapid man-machine communication.

Software

The programming package is written in Assembler Language and Fortran IV. Of course, an overlay structure has been developed to accomplish the following different functions. Four-letters commands, on the computer typewriter console, allow the operator to select the desired step, or to repeat several times the same step, or to skip phases without stopping the main program and beginning the execution again. Some of the utilized phases are of general interest and utility and have been described elsewhere (Dimedio and Fontana 1974).

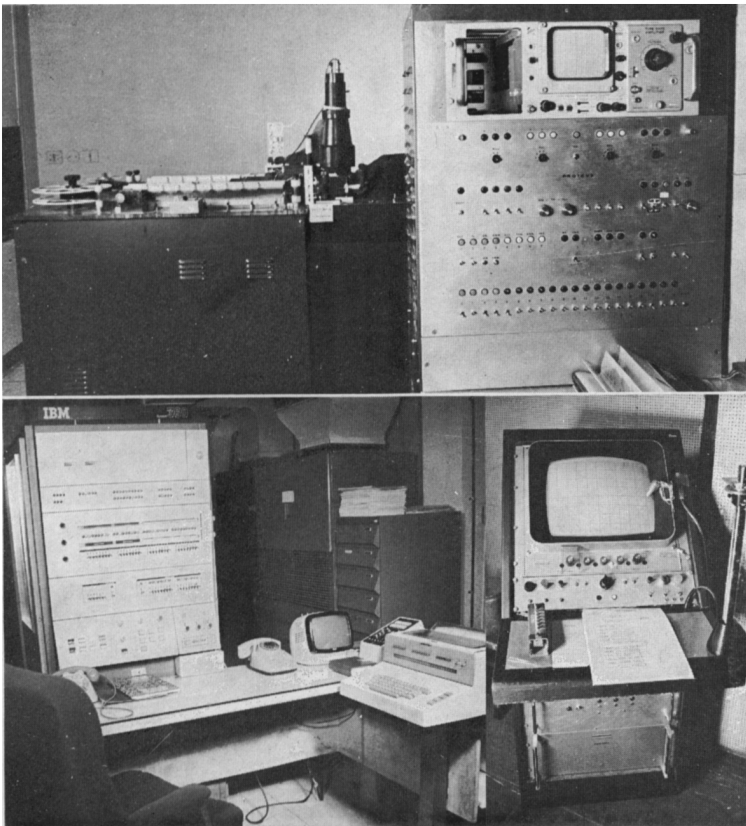


Fig. 2. Current equipment for automated chromosome analysis at CNAF: (a) flying spot scanner 'PROTEUS' for film digitizing; (b) IBM 360/44 computer; (c) display with interrupt switches for man-machine communication.

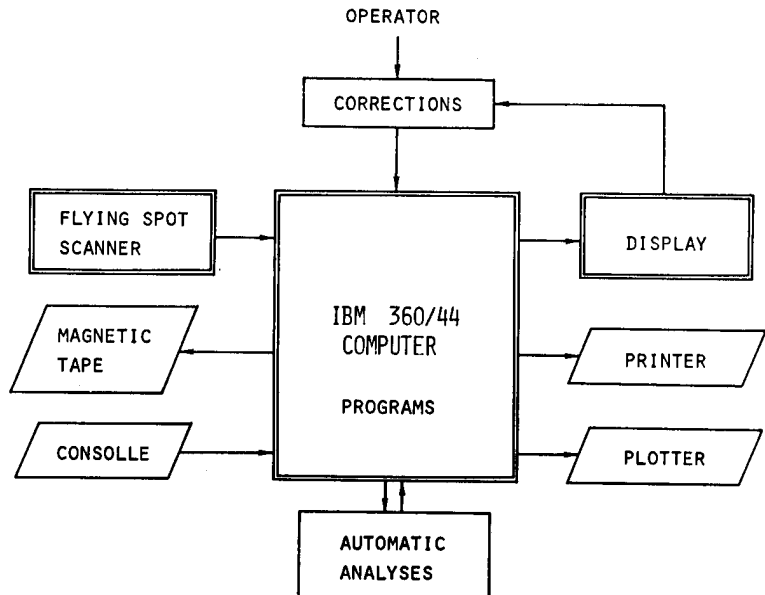


Fig. 3. Hardware of the system.

DATA ACQUISITION

This phase consists of the following main steps, connected with software block structure:

1. *Start*: the operator enters the main program, with common areas, some basic subroutines and initial conditions, and centers the first frame of the 35 mm film reel. Executable 4-letters commands list appears on the typewriter console; all different names are dummy.
2. *Film Advance*: with 'simu' command, the film advances to the requested frame by whole frame increments.
3. *Scanning*: with 'load' command, the computer actuates the scanner channel and allows scanning. The words, with coordinates and densitometric data of each point into the selected rectangle, fill up the free area of memory (buffer of 20 K word-core). When half-buffer is full, data elaboration begins and continues together with acquisition. This on-line elaboration consists of: (a) verifying spot coordinates; (b) identifying whole image row-vectors, divided by a standard recognition value; (c) packing 4 close density values in a word; (d) transferring them to a temporary array; and (e) constructing grey level histogram. When an array is full, its contents are transferred to a magnetic tape as an elementary block of the whole picture. On-line elaboration needs shorter time than scanning, so the flying spot never waits for the computer, but the computer waits for flying spot data, optimizing the time in this phase.

INTERACTION STEP

The 'disp' command actuates the display channel to edit the whole picture with the threshold grey level obtained by the analysis of grey level histogram and to visualize only the spots with a density higher than the surrounding background. (See Technical Notes, 2.) By three different symbols and their arrangement, repetition and overlapping, six well recognizable levels can be visualized on the monitor screen (Fig. 4).

On this picture, the operator can fix the boundaries of the part of the image holding interesting patterns, because the coordinates of the cursor can be transmitted to the computer under the computer-interrupt mode. After this operation, only the internal spots of this rectangle can be visualized. Moreover, it is possible to select any portion of the image to display its enlargement and act on it with the same techniques as on the whole picture. When the image is displayed, it is possible to operate in order to avoid troubles that may be present in the metaphase field, preventing its employment in an automatic analysis. Such occurrences are the following:

1. An error in the threshold maximum value determination. In any instant of the interactive phase the operator can modify threshold maximum value on console.
2. A nonhomogeneous threshold value. By means of a command on console, it is possible to assign a different threshold value to various portions of the image. However, this event is not frequent in our spreads and we prefer to reject such metaphases.
3. Touching chromosomes. The operator can separate any touching chromosomes in the picture equalling some middle spot values to threshold value. This is possible either modifying a single spot positioning the cursor on it, or modifying all the points situated in a strip as large as scanning rate (Fig. 5).
4. Fragments of chromosomes. Chromosomes can appear as two fragments, when a part has a density less than, or equal to, threshold maximum value. This occurrence is more frequent with banded chromosomes because intrinsically they alternate dense and bright zones. The two fragments of a chromosome may be connected with a line changing densitometric profile along the chromosome axis as less as possible. This line is formed by points of density equal to the value of the threshold plus one. As in touching chromosomes, the operator can choose to modify only one point every time or all the points situated in a little strip individuated by the cursor. Fig. 6 shows the effect of this interference.
5. Artifacts of same size as chromosomes. While it is easy to distinguish automatically little artifacts and large nuclear bodies, patterns as large as chromosomes can produce remarkable errors. Therefore, it seems convenient, in the interactive phase, to cancel the patterns producing ambiguity. For this purpose, it is sufficient to direct the bright spot to any spot of the artifacts. The suitable switch causes the cancellation of all connected points (Fig. 5).

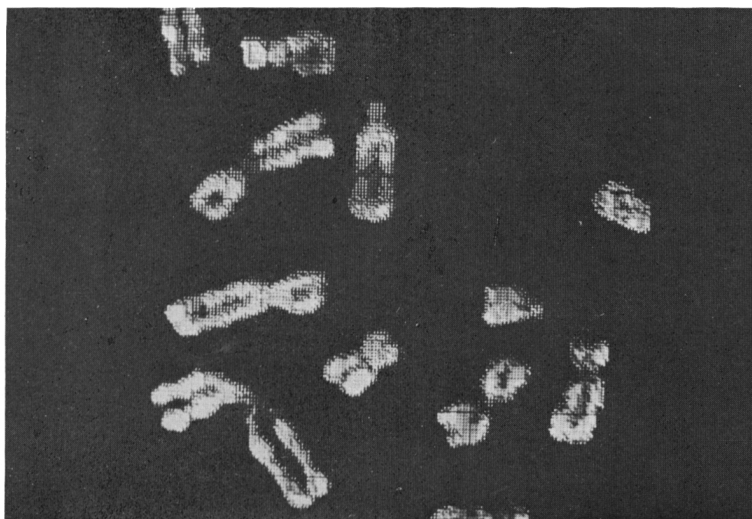


Fig. 4. Display image of chromosomes of a metaphase.

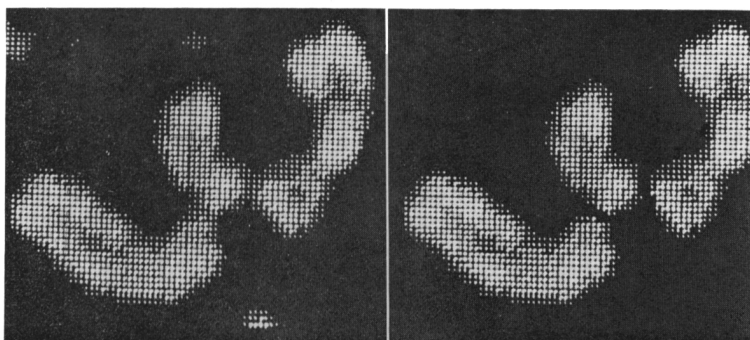


Fig. 5. Touching chromosomes separation and small artifacts cancelling.

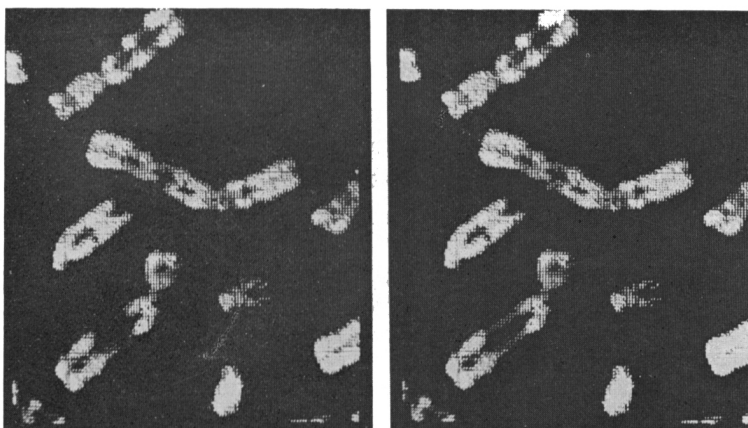


Fig. 6. Connection of chromosome fragments:
 (a) chromosome fragments caused by a very high threshold value;
 (b) connection of these fragments.

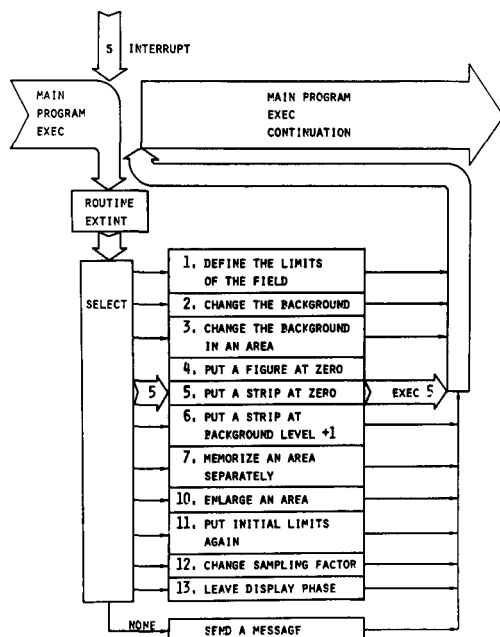


Fig. 7. Scheme of all subroutines set up to execute interactive interventions.

6. Bent and overlapping chromosomes. Particularly difficult chromosome images, as spatially bent or overlapping chromosomes, may be subtracted to automatic analysis and visualized near classified chromosomes in the final step of the procedure, leaving the analysis and the classification to the operator. As in the case of nonhomogeneous threshold value, we prefer to reject such metaphases, which are rare in our slides, and begin data acquisition of another frame.

The effect of every interference produced by the bright spot and the different switches can be immediately shown on the monitor screen to be evaluated and, if needed, modified.

A scheme of all subroutines set up to execute interactive interventions in the phase of metaphase selection is shown in Fig. 7.

CONCLUSIONS

By means of a combined and selective interference, as we have reported, it seems possible to avoid almost all the troubles which make the metaphase automatic analysis difficult or impossible. Since these interferences are made on the digitized spreads, both images directly obtained from a microscope, or those obtained from a microphotography, are suitable for this purpose.

It is necessary to point out that all interferences are not always necessary and that it is possible to find metaphases automatically analysable without any interference.

It is suitable to decide in which way to interfere on the images by evaluating the system performances and efficiency. In fact, the interferences allow to analyse a larger number of metaphases, but require more time.

If a specific image is particularly interesting or significant to the cytogeneticist, by means of our system it is possible to analyse it in spite of technical defaults.

In conclusion, the possibility of interfering during selection of metaphases is not only fundamental in increasing the reliability of the system, but makes it more flexible and adaptable to the unavoidable qualitative differences that spreads always present.

TECHNICAL NOTES

(1) Device features, in the configuration we used, are: (a) scanning rate $44 \mu\text{m}$ (the same for both axes); (b) scanning speed $1.56 \mu\text{m}/\mu\text{s}$, slow enough to reduce tail effect (tail energy lower than 1% of the total energy); (c) scanning period $28 \mu\text{s}$, of which $16 \mu\text{s}$ are used for the measure. This time corresponds to a displacement of $25 \mu\text{m}$, equal to the spot diameter, and allows on the photomultiplier a number of electrons large enough to contain signal/noise ratio within 1% (calculation made at INFN/CNAF on the basis of experimental data collected at CERN); (d) utilisation of a fraction (20%) of the incident beam as measure signal reference, in order to obviate phosphorus unavoidable brightness differences in the various points of the tube, particularly on the borders, and all other proportional troubles. These features make significant signal discrimination in 64 grey levels, as it is necessary in digitizing banded chromosomes' images.

(2) The RCT used for the data visualisation is not a memory tube, nor has it buffer to its disposal in order to memorize displaying data. Therefore, the program reserves a computer memory area to held data which must be cyclically sent to the tube and obtain visualisation refreshing. A routines chain selects points with an optical density exceeding background level from magnetic tape, calculates their coordinates in the display reference system and places it in a word core memory together with a code number representing the optical density level. Data transfer to the display, by the real-time channel, occurs both during buffer's filling, and later on, while the computer is engaged in other functions. The very high data number and the limited memory space reserved to the display makes a data sampling necessary, so that, when the whole field must be visualised, only a point every four (or every nine) is picked up. Therefore, displayed images don't held the whole acquired information, neither in number of points nor in optical density levels. However, resulting images are detailed enough to allow easy interpretation, and enlargements containing more information are always possible. The operator can always interfere on memorized data by selecting the right program preestablished for the various types of intervention foreseen in the scheme of Fig. 7, localising this intervention by a light pen.

(3) The programs referred to in the present study are all available at the National Center for Photogram Analysis, Bologna, Italy.

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RIASSUNTO

Sistema Interattivo per l'Analisi Automatica di Metafasi Umane con Bande R

È stato messo a punto un sistema interattivo per l'analisi automatica di immagini di metafasi umane e per lo studio dei modelli di bandeggiatura lungo i cromosomi in bande R. In questa prima parte sono presentati l'hardware e il software del sistema e descritte la fase di acquisizione dei dati da negativi microfotografici e le procedure interattive che permettono di intervenire sui dati-immagine in modo da rendere analizzabili automaticamente il maggior numero possibile di immagini. Gli interventi più significativi sono il cambiamento del valore soglia del fondo, la separazione di cromosomi a contatto, la connessione di frammenti di cromosomi, la cancellazione di artefatti e la designazione di cromosomi che necessitano un trattamento particolare.

RÉSUMÉ

Système Interactif pour l'Analyse Automatique de Métaphases Humaines avec Bandes R

Un système a été mis à point pour l'analyse automatique des métaphases humaines à partir de microphotographies de chromosomes en bandes R. Il s'agit d'un système interactif dans lequel un opérateur peut intervenir sur les données-image des métaphases digitalisées, afin d'analyser automatiquement un bon nombre de métaphases. Dans cet article le hardware et le software du système sont décrits, ainsi que les opérations interactives les plus importantes, notamment: désignation manuelle de la valeur du fond, séparation des chromosomes à contact, réunion de parties du même chromosome séparées par accident, effaçage des erreurs, désignation des chromosomes qui ne sont pas analysables automatiquement.

ZUSAMMENFASSUNG

Interaktives System zur automatischen Analyse der R-Band-Metaphasen beim Menschen

Beschreibung eines interaktiven Systems zur automatischen Analyse menschlicher Metaphasenbilder und zur Erforschung der Modelle in den R-Band-Chromosomen. In diesem ersten Teil werden *hardware* und *software* des Systems beschrieben, die Datenerhebung aus negativen Mikrophotographien und die interaktiven Verfahren, die eine automatische Analyse der meisten Bilder ermöglichen. Diese Verfahren sind: Wechsel des Hintergrundschwennwertes, Trennung der sich berührenden Chromosome, Vereinigung von Chromosomen-Fragmenten, Beseitigung der Flecken sowie die Bestimmung der Chromosomen, die einer besonderen Behandlung bedürfen.

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