



# **Clinical Cytometry and Histometry**

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## DISTANCES BETWEEN TWO CHROMOSOMES IN INTERPHASE NUCLEI AS DETERMINED WITH DIGITAL IMAGE ANALYSIS.

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In the interphase nuclei the individual chromosomes are located in distinct areas: they are not spread over the whole nucleus (1). Recently it has become possible to stain certain interphase chromosomes with specific markers (1,2). This suggests to determine distribution functions of distances between certain chromosomes by investigating many nuclei (2).

After staining two chromosomes with specific markers, two separate colored areas can be recognized in the nucleus. Now it is possible to analyse the distances between these areas. In this case only the projection of the nuclei is examined.

Due to the large number of nuclei the manual investigation is time consuming. An approach to solve the problem with digital image analysis is presented hereby.

The digitized picture of the cell preparation, which can contain several nuclei, is presegmented with a threshold selection method (3). Then the nuclear edge in the single segments is identified after transforming the segments into the polar-coordinate-system with the centre of the nuclei as origin (4). Finally the locations of the stained chromosomes are determined.

By investigating a large number of nuclei it is possible to perform a statistical analysis of the distance between two chromosomes or the distances between one chromosome and the centre of the nuclei.

It is assumed that the nuclei have the shape of ellipsoids or cylinders with an elliptic basis. Therefore the distribution of the axes propo-

tions  $B/A$  ( $B < A$ ) of the nuclei of the preparation is determined by adapting ellipses to the projection of the nuclei.  $A$ ,  $B$ ,  $C$  are the main axes of the ellipsoids and  $C$  is parallel to the optical axis.

In order to get the theoretical curves (distances in case of a random distribution), the distribution functions for a) ellipsoids or b) cylinders with an elliptic basis with the same axes proportions as the nuclei are calculated by using a Monte-Carlo-procedure ( $N=10000$ ). The distribution functions do not depend on the length of the  $C$  axis.

Two curves are determined:

a) The distance between the projection of randomly distributed points and the centre of the ellipsoid or the cylinder (Fig.1).

b) The distance between the projection of two randomly distributed points in the ellipsoid or the cylinder (Fig.2).

These theoretical curves are compared with the experimental curves (310 nuclei of amnion cells, chromosome 15 labeled) as obtained by digital image analysis. Fig.1 and 2 show both curves.

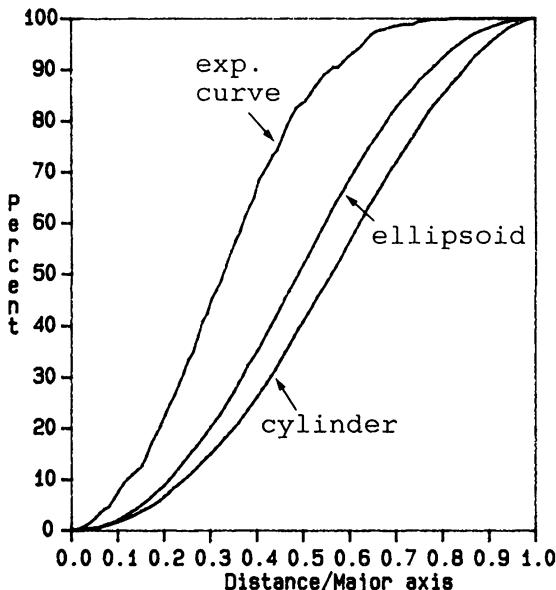


Fig.1 Distribution of distances between the projection of one chromosome and the centre.

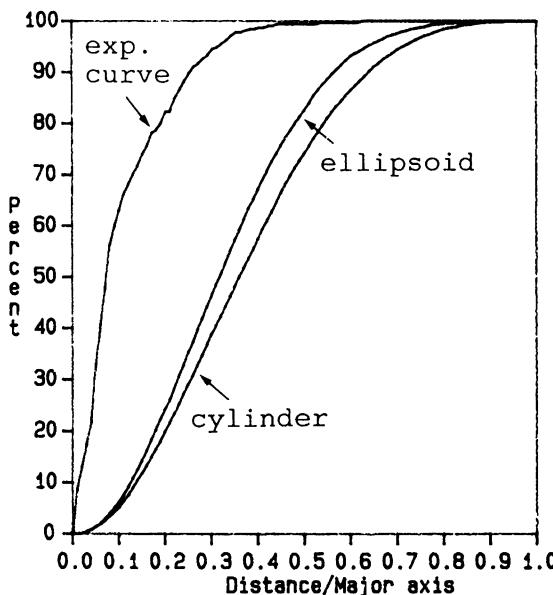


Fig. 2 Distribution of distances between the projection of two chromosomes.

In both cases, a large deviation of the experimental curves from the theoretical curves was found, indicating a non-random distribution.

The experimental curves were obtained by digital image analysis of camera lucida drawings of the specimen displaying the edge of the nuclei and the labeled sites, respectively.

Digitization and analysis was performed on a VAX 11/780 computer. It took the computer two hours to investigate the 310 nuclei; manual investigation had taken several days.

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