Zoological Studies 34, Supplement I (1995)

Zoological Studies

Measurement of Distances in Three-dimensional Dual-colour Confocal Images

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For the quantitative analysis of the spatial relationship between different biological structures in the same preparation, dual-colour confocal microscopy (Carlsson, 1990) has proven to be a successful technique. The spatial relationship between different objects in a two-colour image can be quantified in several ways, depending on the biological information that has to be abstracted from the image. Measurement of co-localization of objects is one of the relevant quantities that can be determined. The large number of methods to quantify co-localization (Ackner et al., 1991; Manders et al., 1993, Wansink et al., 1994) illustrate that each application needs its individual method. An other important quantity of three dimensional dual-colour images containing a large number of spots is the average distance between nearest neighbouring spots. We have developed an image analysis method to measure the latter quantity. We will present this method and show how it can be applied on dual-colour confocal images of cells that were double-labelled with IdUrd and CldUrd (Manders et al., 1992; Manders et al., submitted) to investigate the dynamics of DNA replication (Fig. 1).

To calculate distances between multiple spots in dual-colour images, an accurate determination of the 3-D positions of these spots is essential. The position of the centroid, as an estimator for the position of a spot, can be calculated on the basis of all voxels that belong the domain of the spot. For this calculation a domain that defines which voxels belong to the spot must be delimited (defining a boundary). To create a boundary for a domain we developed a 3-D image segmentation procedure: the Largest Contour Segmentation (LCS) (Manders et al., submitted). By means of this procedure the position of each spot can be determined accurately and automatically (Fig. 2).

After applying this LCS procedure on both colour-components of a dual-colour image (in the sequel we will mention these components as the red and the green component) and the determination

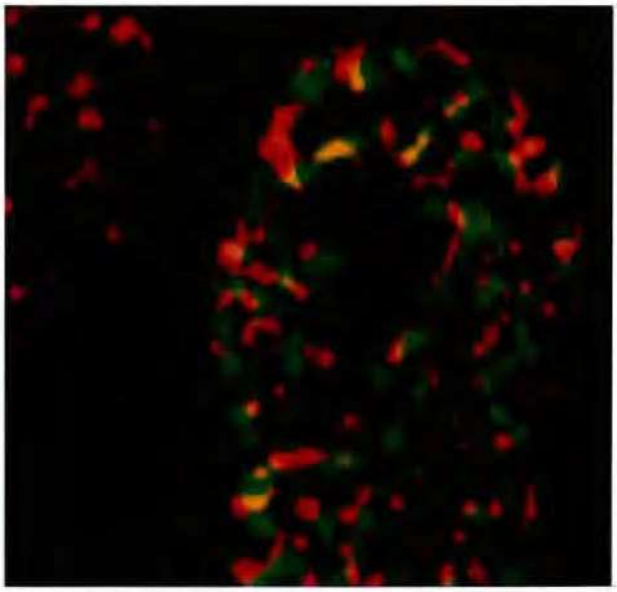

Fig. 1. Preprocessed optical section through the center of a Chinese Hamster cell nucleus, pulse-labelled first with IdUrd (red) and with CldUrd (green) 40 minutes later. After the application of the described method, we measured that the average distance between neighbouring red and green spots is 0.4, μm.

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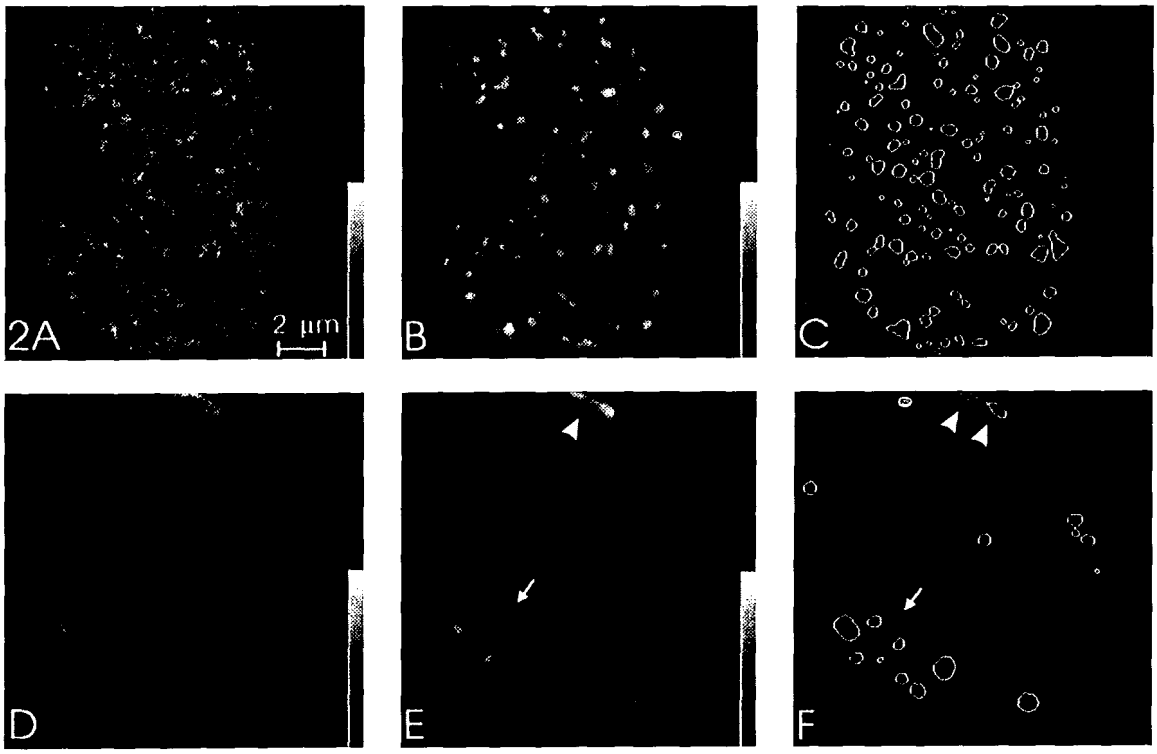

Fig. 2. An optical section through the middle of an early (A) and a late (D) S-phase nucleus as recorded by the confocal microscope. The same 2-D sections (B) and (E) after 3-D image restoration and after 3-D image segmentation (C) and (F). Using the LCS segmentation procedure most domains are detected and their boundaries are defined. However, some are missing (arrows in E and F) and some domains are split into two separate domains (arrowheads in E and F).

of the positions of the spots, the distance between each red spot and each green spot, defined as the distance between their centroids, was computed. Subsequently, pairs of nearest (red-green) neighbours were indicated. A red and a green spot were defined as being paired when there is no other red domain nearer to the green spot and no other green spot nearer to the red one. In that case the inter distance of the pair was listed.

When the average inter-distance of the paired domain is large relative to the accuracy of measurement, the interpretation of the data is simple; the average distance is a direct estimator for the distance that separates the paired red and green spots. When, however, this distance is of the order of the size of the statistical error of measurements, a geometric effect spoils this estimator. The influence of this effect was estimated statistically after which a correction of the data was performed.

This study was supported by the Netherlands organization for Scientific Research (NWO) and SPIN, project 3-D analysis.

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