

NEW 3D COMPUTATIONAL TOOLS TO EVALUATE THE SPATIAL ARRANGEMENT OF POTENTIAL TRANSLOCATION PARTNER GENES IN FIXED NUCLEI

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The spatial configuration and quantification of chromosomes and genes is important to evaluate the nuclear organization and dynamics [1]. Based on the surface rendering of confocal microscopy data, the computer program Kankan has been developed by us to describe and quantify the 3D positions of genes and chromosomes in fixed nuclei, to measure distances and angles between the nuclear entities (Figure 1) [2].

One application of these computational tools is to describe the position of two genes *MLL* (11q23) and *AF9* (9p22). The *MLL* gene on chromosome 11, band q23 has over 50 different translocation partners, one of which is *AF9* gene on chromosome 9, band p22. The t(9;11)(p22;q23) is the most common in *de novo* AML (acute myeloid leukemia) and t-AML (therapy related AML)[3,4]. Between 1-15% of cancer patients develop a form of therapy related leukemia[3].

By calculating the geometric centers of each gene and the nucleus, we are able to define the exact 3D position of a gene within an interphase nucleus and in relation to another gene. If the genes *MLL* and *AF9* are positioned in close proximity, the encounter of pre-damaged DNA, false repair, and the potential for the translocation is increased. Close proximity of two genes might therefore explain the frequency of certain translocations observed in cancer patients. With our investigation we will gain more insight in the spatial organization of *MLL* and *AF9* in interphase nuclei, and therefore understand translocations and nuclear organization in more detail.

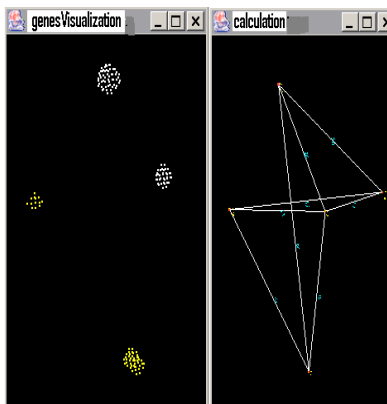


Figure 1. The qualification and 3D visualization of distances and angles among the genes in one representative fixed nucleus. Left: the surface-points of 2 alleles of gene *MLL* (yellow) and 2 alleles of gene *AF9* (white) were extracted from the 3D-reconstructions of confocal image stacks using Amira 2.3 (TGS). Right: the distances (white lines, the values are shown as blue) and angles around the geometric center (red points) of genes represented by program Kankan. The geometric center (c_x, c_y, c_z) is defined as:

$$(c_x, c_y, c_z) = \left(\frac{1}{n} \sum_{i=1}^n x_i, \frac{1}{n} \sum_{i=1}^n y_i, \frac{1}{n} \sum_{i=1}^n z_i \right).$$

Here, n is the total number of points extracted from one gene, and x_i , y_i , and z_i are the coordinates of the extracted points. The calculation based purely on the geometric centers ignores the spatial extensions of the considered genes.

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