Quantitative 3-D Texture Analysis of interphase cell nuclei.

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Abstract

In order to investigate the spatio-temporal structure of chromatin in interphase nuclei we present two 3-D texture parameters based on the Greyweighted Distance Transform that quantify the accessibility and the homogeneity of a nucleus.

Results of experiments on computer generated textures show that these texture parameters are shape independent.

In section 4 we propose to apply the Grey weighted Distance Transform (GDT) on the regions of interest (cell nuclei) in the CSLM images. From the transformed results we extract two texture parameters in section 5. The behavior of these parameters as function of the cell cycle will be studied. To analyze the dynamics of these parameters we have studied them as functions of a range of artificial random distributions that gradually change in texture. Results are shown in section 6.

1: Introduction

The spatio-temporal structure of the chromatin and chromosomes within cell nuclei is an important area of research in cell biology. Little is known about the functional relevance of the position of genes and chromosomes. Expression of genes may not only depend on molecular regulatory units, but the 3-D spatial organization of the genes and the chromatin within the cell nucleus may also be of importance.[2, 3]. Then, we expect to find preferences in gene position, chromosome shape and chromatin distribution, depending upon cell function and cell cycle phase. Research focussed on mitotic phases has been conducted by Oud [4].

The focus of this paper is the interphase of the cell cycle. We have used mithramycin and propidium iodide staining to image the complete chromatin distribution within cell nuclei using fluorescence Confocal Scanning Laser Microscopy (CSLM) [1]. CSLM imaging enables us to investigate the 3-dimensional structure of chromatin distributions.

In the next section we will present our biological objective and hypothesis. In order to verify this hypothesis we are developing a methodology for measuring relevant features of chromatin distributions. Different phases of this methodology involve the restoration and segmentation of the images. These will be discussed briefly in section 3.

2: Objective, hypothesis and definition.

We aim at modeling and studying the spatio-temporal distribution of the chromatin by means of texture analysis. We want to determine if there are structural changes of the chromatin between the different stages (G0, G1, S and G2) of the interphase.

We assume that sites (e.g for gene expression or DNA replication) within the nucleus have to be accessible from the outside while other sites are "inactive". These accessibility patterns supposedly depend on the functional state of the cell.

In this study, we define the accessibility of a point within the nucleus to be inversely proportional to the minimum quantity of fluorescent material encountered while traversing from the border of the nucleus through the chromatin to reach this point. This means we only take into account labeled material (in this case DNA).

3: Methodology

CSLM images have been deformed during the acquisition process by several factors.

Due to the optical properties of the CSLM the optical resolution is about four times lower in the axial direction. Using foreknowledge about the optical properties of the moving scanning table CSLM we can estimate the Point Spread Function (PSF) [9]. This PSF is then used

for iterative Tikhonov-Miller restoration of the images, increasing the optical resolution.

If we want to do quantitative analysis on the images it is essential to correct the images for absorption and reflecting effects on the excitation and emission light. They cause the signal to weaken as the fluorochromes lay deeper in the specimen. Another effect that can be prominent in fluorescence microscopy is bleaching of the fluorochromes. By scanning the deepest planes first these effects are partly compensated. Further restoration techniques depend upon the dominant effect [5]. Extraction of the accessibility patterns by applying the GDT emphasizes the importance of the restoration of these effects, due to the integrating properties of the GDT (see section 4). In the following sections we assume adequate restoration has taken place.

3-D Segmentation of the chromatin distributions is achieved through a split, merge and group technique [7].

4: Calculation of accessibility patterns.

The calculation of the accessibility (as defined in section 2) can be interpreted as an arrival time problem. A 2-D example may clarify this statement. If we observe a 2-D distribution within an object we can look at this distribution as a 3-D landscape. When we start flooding this landscape the character of the landscape will determine the arrival time of the rising water level.

In 1968 Rutovitz [6] introduced the Grey weighted Distance Transform (GDT) in which the distance of a point to a set of reference points (the sources) is calculated as the lowest path sum of grey values (i.e. the lowest of sums over all possible paths from that point to the sources). Verbeek [8] showed that the GDT can be used for the solution of the eikonal equation. (The eikonal equation is used in optics and acoustics to connect local front arrival time with the local refraction index).

We propose to use a 3-D GDT as a matter occlusion detector. Parts of the nucleus that are relatively empty of DNA can be shown to be easily reachable from the outside or not. The algorithm corresponds to the first (cost accumulation) stage of the uniform cost A*-algorithm using 26 connectivity with an appropriate metric.

5: Definition of the texture parameters.

The GDT is a non-reducing transformation. For an (automated) interpretation of the accessibility patterns it is necessary to reduce the data to a few parameters that contain the relevant information. Thus a choice of which information to extract has to be made. In this first attempt at 3-D texture analysis of cell nuclei we restrict ourselves to global parameters.

The first texture parameter we introduce is an indicator for the overall accessibility (as defined in section 2). :

$$g = \sum_{i \in \Omega} \psi_i \quad (1)$$

where ψ_i indicates the GDT value of voxel i , which is a member of the set Ω representing the object in the image.

The range of g is related to the amount of matter (M) in the object, its (discrete) volume (V) and surface area (A) and the (smallest) sampling distance. In order to have a texture parameter which is scale invariant to these variables a normalization of g is imperative. The minimum of g (indicating maximal accessibility) is:

$$g_{\min} = M d_l \quad (2)$$

d_l is the smallest sampling distance between two adjoining voxels. This minimum is shape independent.

Aside from the variables mentioned earlier it is clear (see section 4) that the overall accessibility of an object is highly shape dependent, in other words the accessibility depends on the number of object voxels that are connected to the background. Shape information of an object can be easily expressed by a "surface area over volume" ratio. We are interested by the accessibility pattern given a certain object shape. Therefore an attempt at normalization for shape is called for.

Given an arbitrary object it is very difficult to find a maximum for g , due to very local integration performed by the GDT. An extensive search algorithm would be needed to cover all distribution possibilities.

In the case of a (digitized) sphere it can be shown that the maximum of g is reached when all matter is evenly distributed along the surface of the sphere. The shell value can be easily computed:

$$g_{\max \text{ sphere}} = g_{\text{shell}} = \frac{M V}{A} \quad (3)$$

with A defined as :

$$A = \sum_{i=1}^k \frac{n_i}{d_i} \quad (4)$$

where k indicates the number of possible distances d_i between two neighboring samples. (In the case of an in 3 dimensions equally sampled image k is 3 with the discrete approximations of $d_1 = 1$, $d_2 = \sqrt{2}$, $d_3 = \sqrt{3}$. In the case of typical CSLM images $k = 5$). n_i is the number of voxels on the object surface that have a distance d_i to the background. This is not the best approximation of the "real" surface area of a sampled object. It is consistent

figure 1) Computer generated textures and their corresponding GDT. Left shows textures used in experiment 1 for the sphere. On the right textures in the object "double" are shown.

with the metric used in the GDT and is therefore the correct discrete surface area for normalization purposes.

For more or less spherical objects (like nuclei) the shell value is a good estimate of the maximum. Thus, we define the normalized texture parameter G :

$$G = \frac{g - M d_l}{M \left(\frac{V}{A} - d_l \right)} \quad (5)$$

A value of 1 indicates a highly inaccessible nucleus, 0 indicates maximal accessibility.

We propose a second textural parameter to express the difference in accessibility of the actual matter distribution and a homogeneous matter distribution within a given object:

$$d = \sum_{i \in \Omega} \left| \psi_i - m \delta_i \right| \quad (6)$$

m indicates the mean mass per object voxel and δ_i is the regular distance transform value of each object voxel. In the case of a homogeneous distribution the GDT is reduced to a regular distance transform, multiplied with m . Thus the minimum of D is 0 by definition. We scale D to the value obtained when all mass is concentrated in one voxel (maximal accessibility):

$$D = \frac{d}{M \left(\frac{1}{V} \sum_{i \in \Omega} \delta_i + d_l \left(1 - \frac{2}{V} \right) \right)} \quad (7)$$

6: Experiments on artificial textures.

To study the behavior of the texture parameters G and D we have conducted two sets of numerical experiments on two artificial objects. The first object "sphere" is a 3-D sphere with a 25 voxel radius in an image of 64 x 64 x

64 voxels. The second object "double" consists of two (corrupted) 15 voxel radius spheres with the centerpoints 16 voxels apart in the y-axis in an image of equal size.

In a first experiment we have filled an increasing percentage of the object volumes with voxels of identical grey levels. The remainder was set to 0. Each percentage experiment was performed 10 times with different realizations using a uniform random filling algorithm. A total of 780 measurements were made on the objects.

For the second experiment textures of 25% and 50% filled objects were generated as in experiment 1. These textures were then smoothed by a 3-D gaussian filter with increasing sigma. For each sigma 10 realizations of both textures were generated in the two test objects. Total number of measurements is 720. Figure 1 shows textures and the corresponding GDT's used for these experiments.

7: Results

Results of experiment 1 are shown in figure 2. Both texture parameters show monotonous responses as functions of the filled percentage. Results of experiment 2 are shown in figure 3. Typical CV values of the texture parameters were 1-2%. The G normalization is good for both objects, although the object "double" is not spherical. D shows some shape dependence.

8: Discussion and Conclusions

The experiments indicate that the G and D parameters are complementary for the random structures we have used in the experiments. It is possible however, to find distributions which are highly inaccessible and highly inhomogeneous as well (e.g. an shell configuration will also score a large D). Both parameters will be needed for a thorough accessibility evaluation of cell nuclei.

The shape dependency in D is caused by the fact (see sections 4 and 5) that accessibility is a combination of

Figure 4) G and D against increasing sigma of a 3-D gaussian filter for one fill percentage.

both shape and structure. Therefore a homogeneity measure based upon the accessibility assumption will always be shape dependent. Because the objects of interest to our research do not differ greatly in shape (assuming normal tissue samples) this is not a great disadvantage.

Restoration and correction schemes are essential for a meaningful quantitative analysis of the DNA textures, especially when an integrating transformation (like the Grey weighted Distance Transform) is included in the measuring method.

We have presented two texture parameters based on the GDT that can be used to determine the overall accessibility of a texture within a given object and the homogeneity, based on the accessibility assumption.

Both parameters show monotonic behavior on random textures as a function of filled volume percentage and as a function of the size of a 3-D gaussian smoothing filter

Future plans include analyzing cell nuclei that have been sorted in several populations by flow cytometry on

DNA content to observe possible differences in texture during the different stages of the interphase.

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9: References

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Figure 3) G and D against the filled volume percentage for both objects