Spatial Statistics of the Cytoskeleton

Martin Hanf[1,2], Heinrich Walt[3], Thomas Leemann[2], Diethelm Würtz[1]

[1]Swiss Center for Scientific Computing, ETH Zürich
[2]Institut für Biomedizinische Technik und Medizinische Informatik, Universität/ETH Zürich
[3]Forschungsabteilung Gynäkologie, Departement für Frauenheilkunde,
Universitätsspital Zürich

ABSTRACT
The three dimensional structure of the various types of cytoskeletal filaments (CSK) constitutes the cell's shape as well as the interior organization of cell organelles. Since changes in the numerous phases of cells towards neoplastic transformations are known to affect the morphology of the cell it can be assumed that these morphological changes can be quantitatively described by means of the cytoskeleton. Thus the investigation of the structural organization of the cytoskeletal meshwork appears to be a promising approach to develop new quantitative methods for histopathology.
The development of the confocal laser scanning microscope (CLSM), which are experienced since the eighties from merely a concept to a versatile and powerful tool in science and in daily practical use. Together with the availability of specific immunological markers for the CSK proteins, the CLSM opened up the way for the analysis of the 3-D structure of this fascinating cell constituent.
The complex meshwork of the cytoskeleton reveals a wide variety of patterns, demanding an appropriate description to cover its genuine properties for a quantitative analysis. In this paper we show that the CSK can be modelled with spatial point and area processes and present the statistical methodology for its structural analysis.

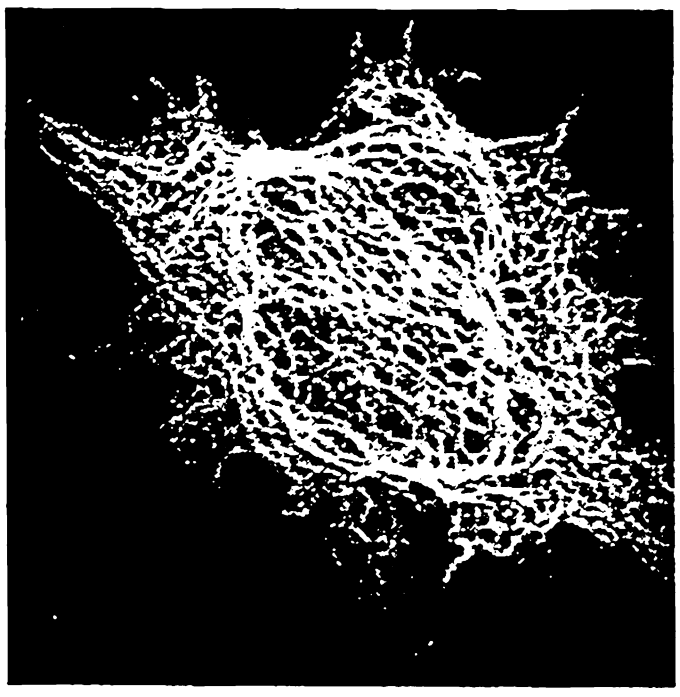

Figure 1: Projection of a CLSM image of the cytoskeleton. Image provided by Prof. Komitowski, Division Histodiagnostic and Pathomorphological Documentation, German Cancer Research Center, Heidelberg, Germany.

GRAPH REPRESENTATION OF THE CYTOSKELETON

The thickness of CSK fibers range from 4 nm (actin filaments), 10 nm (intermediate filaments) to 25 nm (microtubules). Therefore these structures can not be resolved perfectly with confocal laser microscopy, which provides a axial resolution of about 230 nm for a wavelength of 442 nm [1]. Consequently, any direct measurement on the spatial distribution of structures by means of the density of fluorescence dye attached to the CSK fibers can not be regarded reliable. However, whereas the thickness and the number of fibers in a bundle can not be conclusively determined, the length of the filaments and the overall structural organization of the meshwork can serve as a basis for an analysis.

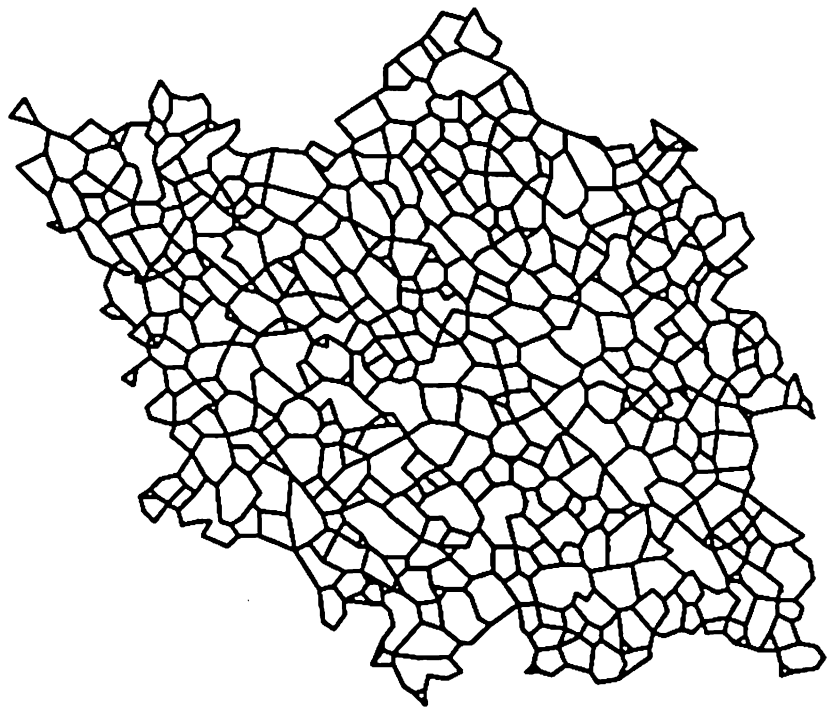

Figure 2: Graph representation of the skeletonized CLSM image (Figure 1).

The topological properties of the meshwork can be described adequately in terms of graph theory, representing the structures as a graph $G(V,E)$ with the sets of vertices V and edges E. Figure 2 shows the graph of the cytoskeleton as extracted from figure 1. Weighting the edges corresponding to their length we yield a *planar*, *undirected* and *weighted* graph.
A variety of properties like the distribution of the degrees of vertices, i.e. the number of edges of a vertex, are now available for classification purposes [2]. Here we like to present an analysis based on the *cycle basis* of the graph, which is defined as the set of *elementary* and *independent* cycles, such that any other cycle can be written as a linear combination of the cycle basis [3].

SPATIAL PROCESSES

Although phenomenological parameters like the degree of the vertices can be easily defined on the graphs, their relevance for the analysis of the CSK is not given a priori. A more fundamental approach is to model the structures of interest and to apply statistical tests to investigate the significance of the properties found. Spatial point and area processes are a natural choice for the modelling of the experimental data, since they allow in principle to incorporate a priori knowledge of pattern formation process of the CSK.

However, before considering detailed and worked out hypotheses on the underlying processes which form the structure of the cytoskeletal meshwork, it is obvious to start with the most basic Poisson process. This process is defined by the following properties [5]:

1.) The number of points in any given set has a Poisson distribution mean.

2.) The counts in disjoint sets are independent.

Thus, the poisson process serves as an idealized representation of complete spatial randomness (CSR).

Point processes are able to model the spatial distribution of the vertices or center points of enclosed areas, but they can not capture the topological properties of the CSK graph. However, using the Dirichlet tessellation, we can transform the point processes to patterns which resemble the CSK graphs and can apply a statistics based on the cycle basis which is particularly sensitive to periodic or regular spatial structures. A detailed introduction and analysis of this test is given in [4].

RESULTS

Figure 3 shows *Ripley's L-Statistics* which is based on the expected number of points within diameter t (in pixel units) around any other point [5].

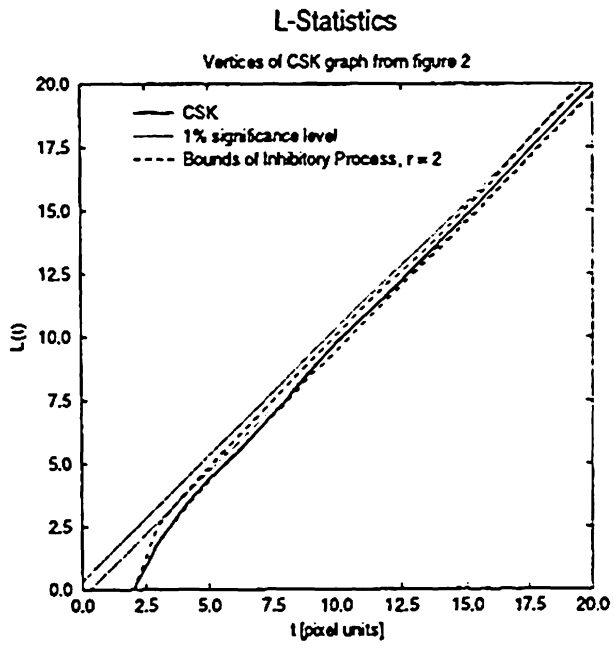

Figure 3: Ripley's L-Statistic for the CSK graph of figure 2.

The experimental data deviates significantly from the CSR hypothesis only for diameters $t < 5$. However, since the vertices of the graph are restricted to an image grid, it is obvious, that vertices can not be closer than 2 pixel units without losing the property of being different vertices. Thus, this deviation has to be regarded as an artefact of sampling process. Considering an 'inhibitory random process' which does not allow two points within the given radius is able to model this artefact.

Figure 4 shows the *Cycle Base Statistics* results for the same data. Though also this test does not show significant deviation from the null hypothesis of complete spatial randomness, some structure in the distribution of the perimeters of the cycles is apparent, suggesting periodicity in the data.

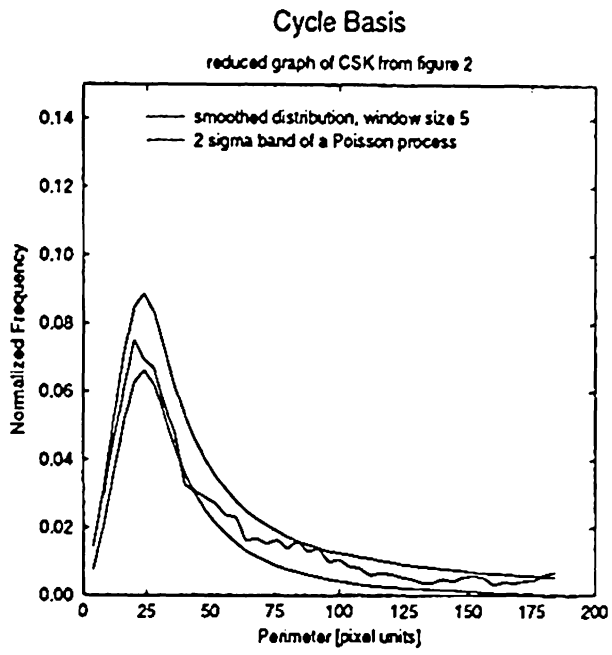

Figure 4: Cycle Basis-Statistics for the CSK graph of figure 2.

CONCLUSION

Both tests show that the data analysed in this study can be modelled by a Poisson process within the chosen significance level of 99%. Deviation from the null hypothesis of a complete spatial random process can be attributed to sampling artefacts due to the image grid.

Although the choice of the significance level is arbitrary, a reliable classification of the cytoskeletal meshwork for diagnostic purposes demands a significant deviation from a random process. According to the given data, the usage of the CSK structures as a basis for diagnosis is a task which has to be performed carefully.

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