Probing filamentarity of nuclear skeletal structures by methods of spatial statistics, mathematical morphology and image analysis

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Correct positioning and spatial arrangement of genes and functional molecular complexes within the cell nucleus is required for the processes of gene expression and genome duplication. This is provided, at least partially, by the nucleoskeleton, comprised of outer lamina and inner nucleoskeletal network. While the structure and composition of nuclear lamina is in many aspects well understood, the molecular nature of the inner network remains elusive despite several decades of scientific effort. Considerable amount of data suggests that it is formed by several skeletal subsystems, including actin-containing structures, lamins, and possibly some other type(s) of intermediate filaments [1, 2].

To assess the molecular architecture of the nucleoskeleton, we performed a screening using various antibodies against potential nucleoskeletal proteins for immunogold labeling either on the surface of ultrathin sections or using the pre-embedding labeling technique for subsequent electron tomography. Gold particles on the ensuing image data form a two-dimensional or a three-dimensional discrete point-like pattern, which can be considered as a realization of a point process in the Euclidean space.

Our first objective of this study is to apply statistical methods of characterization of filamentarity in these point-like patterns. Our second objective is to apply statistical methods of filaments extraction and visualization from these data. We first explore and compare the capability and efficiency of various tools for filamentarity quantification and filaments extraction in the case of a specific protein, the filamentarity of which is firmly established, e.g., vimentin, and then we proceed to explore filamentous structures formed by e.g. actin in the nucleus.

Many standard summary characteristics, which are effective and robust tools for discriminating regularity, clustering and collocalization in uni- or multi-variate spatial point processes are available, but few of them are useful for checking the presence of linear structures. To characterize geometry and topology of point patterns in more detail, a set of morphological functions defined by means of Minkowski functionals was introduced. There are two general ways of how Minkowski functionals may be employed to describe morphology of a point pattern. A Boolean grain model may be associated with the point process by decorating each point with a ball of the same radius. The union set of the covering balls is then studied morphologically, whereby the varying radius of the balls serves as a diagnostic parameter probing the spatial scale of the pattern. As an alternative, the point pattern is smoothed by some suitable kernel to form a continuous random field and its excursion sets are studied morphologically for varying thresholds. Since we may also vary the smoothing scale used in constructing the continuous field in the excursion set approach, the redundancy of having two diagnostic parameters may partially be amended by using the

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Delaunay Tessellation Field Estimator (DTFE), the relevant feature of which is its sensitivity to anisotropic features like filaments. Particular ratios of Minkowski functionals called "Shapefinders" provide us with measures that characterize filamentarity of single objects that arise in the excursion set approach for a high enough threshold. A global method for extracting shape information also from non-convex bodies uses global Quermaß vectors. Multiscale Morphology Filter employing multiscale analysis, which stems from image processing, represents another way of dealing with linearity in point patterns. It is based on inspecting the continuous field created by DTFE with successively diminishing resolutions. The filamentarity is then assessed as a maximum morphological response across the full range of smoothing scales; at each scale the filamentarity is quantified by examining the eigenvalues of the Hessian matrix at each spatial location. Yet another effective method uses local dimension analysis of the point pattern [3, 4].

As to the filaments extraction from a point pattern, multifarious methods have been developed in the context of morphometry of cosmic structure and image analysis. The following techniques turn out to be the most effective ones for our analysis: Minimum spanning tree finding, Curve fitting algorithms, Skeleton method, Beamlet transforms and the use of Candy models [5], (Fig. 1).

Our approach seems to be both functional and effective in that it helps in determining the morphology of nuclear skeletal structures in an objective way. To our best knowledge no similar analysis probing filamentarity of the nuclear skeletal structures by means of statistical methods have been published yet.

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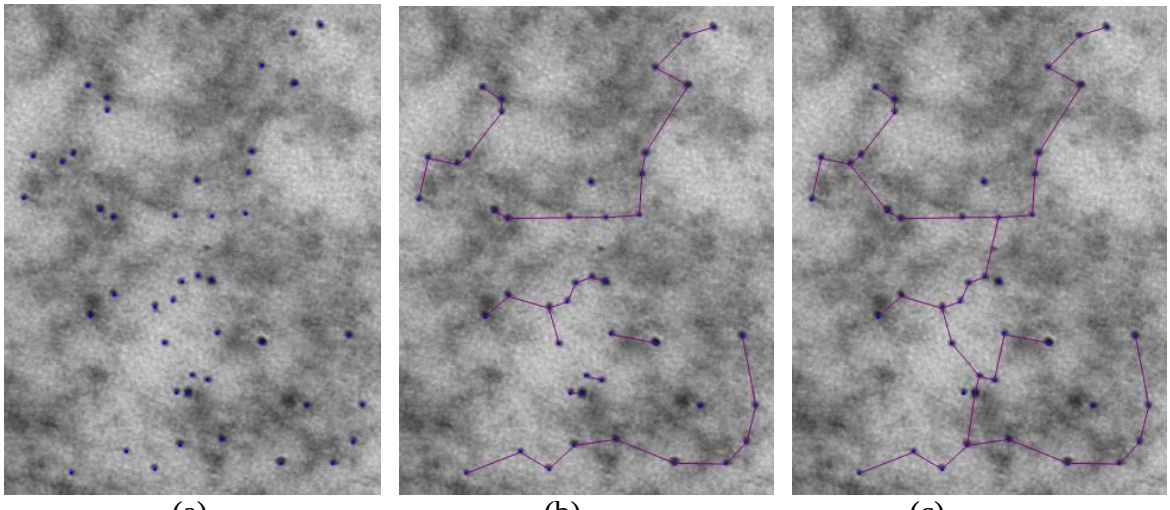

(a) (b) (c)

Figure 1. (a) Pre-embedding labeling of vimentin in HeLa cells. Gold particles size 5 nm, section thickness 150 nm, TEM image. (b) Application of a curve fitting algorithm. (c) A pruned Minimum spanning tree.