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A lymphocyte spatial distribution graph based method for automated classification of recurrence risk on lung cancer images

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ABSTRACT

Tumor-infiltrating lymphocytes occurs when various classes of white blood cells migrate from the blood stream towards the tumor, infiltrating it. The presence of TIL is predictive of the response of the patient to therapy. In this paper, we show how the automatic detection of lymphocytes in digital H&E histopathological images and the quantitative evaluation of the global lymphocyte configuration, evaluated through global features extracted from non-parametric graphs, constructed from the lymphocytes' detected positions, can be correlated to the patient's outcome in early-stage non-small cell lung cancer (NSCLC). The method was assessed on a tissue microarray cohort composed of 63 NSCLC cases. From the evaluated graphs, minimum spanning trees and K-nn showed the highest predictive ability, yielding F1 Scores of 0.75 and 0.72 and accuracies of 0.67 and 0.69, respectively. The predictive power of the proposed methodology indicates that graphs may be used to develop objective measures of the infiltration grade of tumors, which can, in turn, be used by pathologists to improve the decision making and treatment planning processes.

Keywords: Digital pathology, Lung cancer, Lymphocyte detection, Classification, Machine Learning, Graphs, Tumor-infiltrating lymphocytes , MST, Delaunay , Gabriel graph

1. INTRODUCTION

Development and progression of malignant tumors is characterized by interaction with other cells in the tumor microenvironment including infiltrating immune cells, fibroblasts, and endothelial cells.[5,8,25] Different studies have shown that tumor-infiltrating lymphocytes (TILs) are predictive of the response to the neoadjuvant therapy and after the adjuvant chemotherapy.[8,19] These reports suggest that the cancer biology and treatment response is dependent on different degrees of lymphocyte infiltration into the tumor microenvironment, so it may be important to include TILs in the pathology routine evaluation.[8] Unfortunately, there are not standardized, objective, and effective TIL quantification strategies.[3,4,18] Current approaches for grading TIL are highly subjective and show poor reproducibility.[3]

Different authors highlight the importance of establishing standardized methods for reproducible assessment of TILs, so they could be used as a biomarker.[8,18] Development of automatic quantification strategies based on image analysis techniques could aid to this purpose.[3]

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Graphs in Histopathology

Graphs are mathematical constructions composed of finite sets of connected objects that capture global and local relationships via pairwise connections between its members. Their use in the analysis architectural and spatial relationships in histopathology imaging (HI) was first proposed in the late 80s[23] with practical applications being developed by the early 90s[1,17] Due to their ability to quantify and describe the arrangement of structural tissue primitives, graph-based techniques are still a valuable tool used to characterize and analyze histopathologic images.[14–16,21,21]

The earliest attempts in the area favored the more intuitive approach of using nuclei as vertices[17] whereas subsequent approaches leaned towards the use of larger objects extracted from coarser segmentations (such as cell clusters) as nodes[10].[2] Recent literature reviews on HI automated cancer diagnosis,[7] HI analysis,[11] and graph-based methods for HI[21] confirm this trend.

Automated Infiltration estimation

Some works in the literature have attempted to address the problem of automatically estimating the infiltration grade of lymphocytes by means of graphs.[4,12,13] In these approaches, authors start by applying different pre-processing strategies to identify lymphocytes on images. These strategies include Markov Random Fields, sigmoid contrast enhancement, conditional hole filling, adaptive active contour, extraction of Haralick features, the scale-invariant feature transform (SIFT) algorithm, and so on. After locating the lymphocytes, some graphs are built (Delaunay triangle, Voronoi diagram, and minimum spanning tree), and from them different features are extracted to predict the infiltration grade for cancer H&E images. While these works exploit architectural features for predicting the lymphocyte infiltration grade, correlation of such infiltration with the patients outcome was not studied.

This article introduces an automated strategy that exploits topological characteristics of lymphocytes in digital H&E images for predicting recurrence in early-stage non-small cell lung cancer (NSCLC). The method starts by automatically identifying lymphocytes based on some shape, color, and texture features. Next, the positions of these lymphocytes are used as nodes to construct a set of non-parametric graphs based only on the relative positions among them. From these graphs, a set of 18 global graph features was used to characterize the microscopic power fields. Finally, this information is correlated with patient survival in NSCLC.

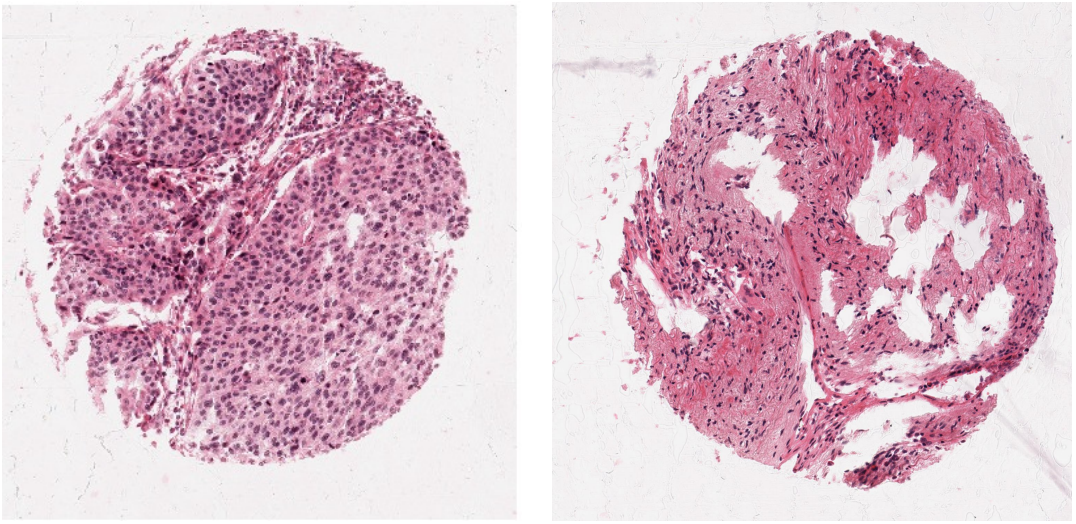
2. METHODOLOGY

2.1 Dataset

This study included samples from a previously reported and well-characterized collection of NSCLC represented in tissue microarrays (TMAs), namely YTMA140 (n=63).[20] This collection includes samples collected independently at Sotiria General Hospital and Patras University General Hospital between 1991 and 2001. Clinicopathological information from patients in the dataset was collected from clinical records and pathology reports. Dataset samples were labeled as *poor* or *good* depending on whether the patient died or not of disease during follow-up, respectively. These designations were used for classifier development and validation.

2.2 Lymphocyte identification

The first step towards an automatic quantification of TILs consists in automatically detecting the existing lymphocytes in a spot. For this purpose, a watershed-based method[22,24] is firstly used to segment image nuclei. In H&E images, lymphocyte nuclei are generally distinguished from other cell nuclei by their smaller size, more circular shape, and a darker homogeneous staining.[4,13] For this reason, a set of texture, morphological, and color features are extracted from those segmented nuclei; then, they are classified as either lymphocytes or non-lymphocytes based on such features.



Good *Poor*

Figure 1. Two samples with different prognosis from the dataset described. The image on the left corresponds to a *good* outcome (patient survived) and the one on the right to a *poor* outcome (patiend died).

2.3 Graph Selection

Once the individual lymphocytes have been detected, it is necessary to code their spatial information in each of the samples of the microarray. In order to do this, a graph is constructed using each lymphocyte's location as a node.

As explained in Section 1, there have been multiple types of graphs used for similar tasks. It is beyond the scope of this work to test and compare all of them. Instead we will limit ourselves to three commonly used graphs: Minimum Spanning Trees (MST), Delaunay, and k-nearest neighbors (k-nn).

A k-nn is a graph in which a node is connected to its k nearest neighbors. Strictly speaking, k-nn should be a directed graph as the neighborhood relations are not symmetrical, *e.g.* node n_i might be one of the k nearest neighbors of n_j but n_j is not necessarily one of the k nearest neighbors of n_i. In this paper we use an undirected graph connecting all neighbors, *i.e.* an edge between n_i and n_j implies that at least one of the the nodes is one of the other's k nearest neighbors.

Delaunay graphs[6] are based on the homonymous triangulation of a set of points/nodes in which no node lies within the circumcircle of any triangle.

Gabriel graphs[9] are a subset of Delaunay graphs in which an edge exists between two nodes V_m and V_n if there is no other node within the closed disc of which the segment V_mV_n is a diameter.

MSTs are connected graphs (a path exists between any two nodes) that minimizes the total edge weight. When using an Euclidean metric, as in the present paper, MSTs are a subset of both Gabriel and Delaunay graphs.

Three of the selected graphs (Gabriel, Delaunay, and MST) have the advantage of not needing any parameter adjustment as they are only based on the relative distance between nodes. Although, also based on relative distances, the third type of graph tested, k-nn, is actually a family of graphs depending on the chosen k.

2.4 Graph Features

A set of 18 well known global graph-based features was calculated for each of the samples. among the different types of Features calculated were:

- Connectedness measure (average degree, average cluster coefficient, number of triangles, number of nodes, number of edges, maximum degree).

- Shortest-path distance related (radius, diameter, average eccentricity).
- Statistical measures of the edge weight (total weight, mean, max, min, max/min ratio, standard deviation).
- Spectral measure (largest and second largest eigenvalue, energy of adjacency).

3. EXPERIMENTAL SETUP

The features calculated from each graph and sample were used to train a supervised boosted ensemble classifier. The purpose of the classifier is to predict, from the lymphocyte's graph topological information, if a patient has been labeled as *good* or *poor*. A *good* label is considered a positive value.

Due to the relatively small number of cases, validation of the presented approaches was carried out using a leave-one-out cross validation on the previously described dataset (see Section 2.1).

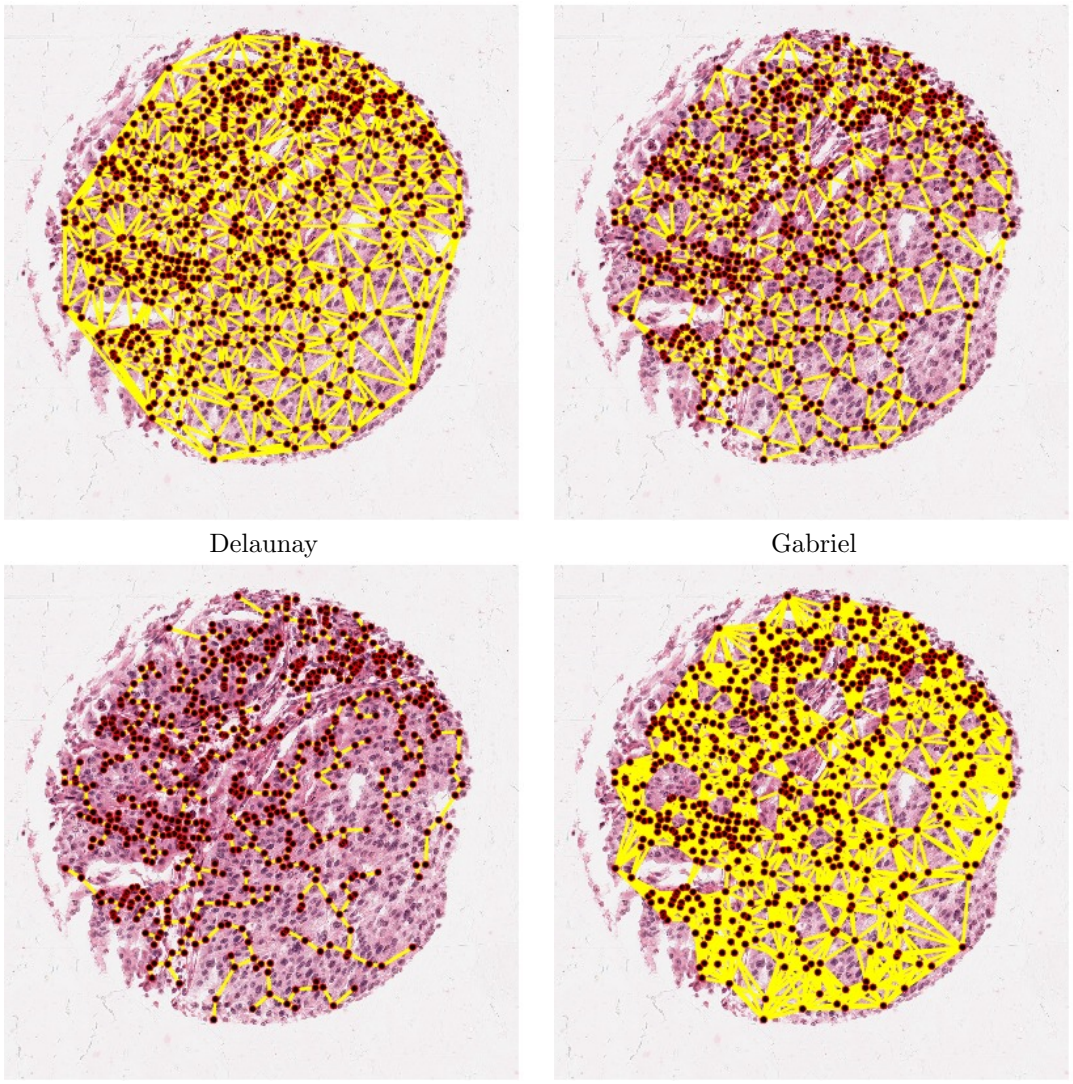


Figure 2. The detected lymphocytes and calculated graphs are overlayed over the spot with the *good* outcome shown in Figure 1.

Graph Type	Sensitivity	Specificity	F1 Score	Accuracy
MST	**0.9375**	0.4	**0.75**	0.6674
K-nn (k=10)	0.7812	0.6000	0.7246	**0.6935**
Gabriel	0.6875	**0.6667**	0.6875	0.6774
Delaunay	0.2188	0.8333	0.3182	0.5161

Table 1. Classification performance measures using a boosted classifier and a leave-one-out cross validation.

4. EXPERIMENTAL RESULTS

The results of the leave-one-out cross validation for each graph type are shown in Table 1. For k-nn only the best result are shown (k=10).

The tested features from most of the chosen graphs show an accuracy above 65%, indicating an actual predictive value well above chance. The only exception is the Delaunay graph, which has an accuracy only slightly above 50%.

The $F1$ score, which measures the harmonic mean of precision and sensitivity, showed good results in MST and K-nn graphs (0.75 and 0.7246, respectively). Results for the Gabriel graph were fairly good (0.6875), whereas Delaunay had a very low score (0.3182).

5. DISCUSSION AND CONCLUSIONS

In this work, we propose a methodology and show how the automatic detection of lymphocytes could contribute to develop objective and precise mechanisms to measure infiltration grade of tumors. This is an additional tool which can be used by pathologists in their decision making and treatment planning.

The best $F1$ results in these tests were found using the MST. It is interesting to note that MST were the sparsest graphs tested, *i.e.* the ones that had the lowest edges to nodes ratio. Sparse graphs have the advantage of having a smaller computational overhead when calculating features. Additionally, sparsity tends to dismiss additional or redundant information which may confound the classifying stage. It should be noted that MST have been traditionally used to find the underlying structure of clouds of points (sometimes called the 'skeleton'), making it a good candidate for automatically detecting topological patterns.

These initial results show a very good performance, particularly taking into account that only the topological information extracted from the position of the lymphocytes was used, *i.e.* lymphocyte features derived from color, texture, size or shape were not used in the classification process. Further work might add appearance features to either improve the classifier or to help create the graph, *e.g.* similar appearance lymphocytes might have a higher probability of having an edge between them.

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