

Statistical analysis of spatial patterns in tumor microenvironment images

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Advances in tissue labeling, imaging, and automated cell identification now enable the visualization of immune cell types in human tumors. However, a framework for analyzing spatial patterns within the tumor microenvironment (TME) is still lacking. To address this, we develop *SpatioPath*, a null-hypothesis framework that distinguishes statistically significant immune cell associations from random distributions. Using embedding functions to map cell contours and tumor regions, *SpatioPath* extends Ripley's K function to analyze both cell-cell and cell-tumor interactions. We validate the method with synthetic simulations and apply it to multi-color images of lung tumor sections, revealing significant spatial patterns such as mast cells accumulating near T cells and the tumor epithelium. These patterns highlight differences in spatial organization, with mast cells clustering near the epithelium and T cells positioned farther away. *SpatioPath* enables a better understanding of immune responses and may help identify biomarkers for patient outcomes.

Immunotherapy has become a mainstream cancer treatment, thanks to positive and unprecedented clinical outcomes against many types of cancers. Recent research in cancer biology has highlighted the importance of studying the (micro-)environment of the tumor (TME), which includes immune cells, stromal cells, blood vessels, and extracellular matrix¹. The TME has a dual ambivalent role: it is crucial for cancer progression and metastasis, but it also contains immune cells that fight against the cancer cells. Interestingly, the spatial distribution of immune cells within the TME seems to be critical to predict patient outcome^{2–4} or evaluate the response to immunotherapy⁵. For example, the infiltration of T cells is commonly associated with good prognosis⁶, and Li et al. recently studied the spatial distribution of CD8⁺ T cells in triple negative breast cancer⁷. Similar studies have been conducted for other kinds of cancers as well: Zwing et al. analyzed the density and association distance of myeloid cells and T cells in colorectal cancer tissue samples, and observed a correlation between the spatial distribution of the cells and the functioning of cytotoxic T cells⁸. Similar findings based on the spatial

organization of immune cells within the TME are discussed in the review paper by Heindl et al.⁹. Recent advances in tissue staining complemented with state-of-the-art imaging¹⁰ have made possible the in situ mapping of the TME of several types of cancer. However, a recurrent image analysis problem is then to reconstruct key structures within the TME as spatial objects either by segmenting structures of interests (such as tumor epithelium, stroma, etc.) or via automatic localization of the different immune cell types using advanced object detection techniques. While such tasks have dominated research in image analysis, it may be said that they only serve as the *hors d'oeuvre* in the grand scheme of gaining a system-level understanding of the immune response during therapy. Equipped with state-of-the-art algorithms which can digitally reconstruct and localize different biological structures at scale, the next generation challenge is to extract robust information to analyze the spatial data and extract biologically relevant information.

From a mathematical perspective, it is first necessary to describe the association between two different populations of already detected

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