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Phenotyping immune cells *in-situ*: Highly multiplexed imaging and analysis of immune subsets in FFPE tissue sections

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Successes with PD-L1 drugs and adoptive immunotherapy demonstrate the efficacy of leveraging the immune system to fight cancer. However, host-tumor interaction is complex and difficult to characterize with immunohistochemistry or flow cytometry. Visual assessment cannot easily determine the phenotype of immune cells *in-situ* and multimarker quantitation in FFPE tissue sections is difficult if not impossible with standard methods. Capturing spatial relationships of immune phenotypes in and around tumor would be advantageous, potentially forming the basis of assays to guide therapy and monitor response. To address this, we present a multi-marker, computer-aided event-counting methodology for determining the phenotypes of immune and other cells in a range of tumor types using a multispectral imaging (MSI) automated tissue segmentation and cell counting approach. A range of protocols on FFPE sections will be shown, including one for melanoma samples, with simultaneous labels for PD-L1, CD8, CD34, and FOXP3, and DAPI, and the other on follicular lymphoma, with labels for CD3, CD69, FOXP3 and hematoxylin. Results demonstrate reliable detection and phenotyping of lymphocytes in heterogeneous clinical samples, high signal-to-background and precise measurement of per-cell expression, and counts and distributions of phenotyped immune cells in both intra- and extra-tumoral compartments. We believe we have demonstrated a new capability for elucidating the intricacies of cancer immune response, for research and potentially for clinical use, with a workflow that is automated by computer and amenable to present practices where results are reviewed by pathologists to assure data quality.

Biography

James R Mansfield is a Scientist with over 25 years of experience in spectral imaging, in-vivo spectroscopy and applied data analysis, directed towards finding of novel optical methods for the diagnosis and monitoring of medical conditions. He is currently the Director of Quantitative Pathology Applications at PerkinElmer where he is the senior application scientist for their multispectral and digital pathology product lines. He is an Associate Editor of the American Journal of Nuclear Medicine and Molecular Imaging, holds 6 patents, has over 50 publications and has served as an invited speaker, session chair and organizer at a variety of international conferences.

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