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Imaging in cancer immunology: Phenotyping of multiple immune cell subsets *in-situ* in FFPE tissue sections

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There has been a rapid grown in the field of tumor immunobiology in recent years as a result of recent successes in cancer immunotherapies, and it is becoming clear that immune cells play many sometimes conflicting roles in the tumor microenvironment. However, obtaining phenotypic information about the various immune cells that play these roles in and around the tumor has been a challenge. Existing methods can either deliver phenotypic information on homogenous samples (e.g., flow cytometry or PCR) or morphologic information on single immunomarkers (standard IHC). We present here a methodology for delivering quantitative per-cell marker expression and phenotyping, analogous to that obtained from flow cytometry, but from cells imaged *in situ* in FFPE tissue sections. This methodology combines: The sequential multi-marker labeling of up to 8 antigens using antibodies all of the same species in a single section; automated multispectral imaging (MSI) to remove the typically problematic FFPE tissue autofluorescence and correct cross-talk between fluorescent channels; and an automated analysis that can quantitate the per-cell marker expression, determine the cellular phenotype, count these cells separately in the tumor compartment and in the stroma and provide high-resolution images of their distributions. We present here three examples of this new methodology: The simultaneous labeling, analysis and validation of CD3, CD8 and FOXP3 multiplexed staining in follicular lymphoma; a CD4, CD8, CD20, pan-CK T_H, T_C and B cell panel in breast cancer; and PD-L1, CD8, CD34 and FOXP3 phenotyping and quantitation in melanoma. Each example will show the application of the multiplexed staining, per-cell quantitation and cellular phenotyping from multispectral images of FFPE tissue sections.

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, which are being used in a wide range of fluorescence and brightfield microscopy applications. Before PerkinElmer he worked at Cambridge Research & Instrumentation, where he helped develop their multispectral imaging systems. Prior to that he worked at the National Research Council of Canada as a research scientist and at several small companies developing non-invasive spectroscopic methodologies. His research has included projects ranging from the objective classification of skin cancer spectra using mid-infrared spectral imaging, to developing methods for the non-invasive determination of the severity of rheumatoid arthritis, to the development of the first of several spectral imaging systems able to map out skin oxygenation levels. 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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