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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 growth 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 auto fluorescence 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 examples of this new methodology: The simultaneous labeling, analysis and validation of CD4, CD8, CD20, PD-L1, Foxp3, cytokeratin and DAPI in breast cancer; and CD8, CD34, PD-L1, FOXP3 and DAPI in head and neck squamous cell carcinoma. Each example will show the application of the multiplexed staining, per-cell quantitation and cellular phenotyping from multispectral images of FFPE tissue sections, as well as methods to explore the spatial distributions of the phenotype cells in and around the tumor.

Biography

Kristin Schmidt studied transcription factors involved in the development of the retina in a neurobiology lab at UCLA's Jules Stein Eye Institute. There, she became familiar with assorted tissue preparation techniques, IHC, IF, microscopy, and laser capture microdissection/microgenomics. In 2005 she joined Arcturus Bioscience and provided tech support for LCM instruments and reagents, troubleshooting issues ranging from harvesting tissue to biomolecule extraction and analysis via microarray, real-time PCR, or next-generation sequencing. In 2006 she moved into a global Field Applications Scientist role with Molecular Devices and in 2010 with Life Technologies where she trained LCM users and distributors all around the world. In 2015 she joined PerkinElmer Inc. as a Field Applications Scientist specializing in Phenoptics – multiplex staining in FFPE tissue, multispectral imaging, and image analysis.

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