

## **Imaging in Cancer Immunology: Phenotyping of Multiple Immune Cell Subsets In Situ in FFPE Tissue Sections- Maciej P Zerkowski- University of Pennsylvania**

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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 6 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 image 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 several examples of this new methodology in breast, lung and head and neck cancers. Each application example will show 6-plex multiplexed staining, per-cell quantitation of each marker and multi-marker cellular phenotyping from multispectral images of standard clinical biopsy sections as well as methods to explore the spatial distributions of the phenotyped cells in and around the tumor.

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