

Imaging in Cancer Immunology: Phenotyping of Multiple Immune Cell Subsets *In Situ* in FFPE Tissue Sections

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ABSTRACT: Cancer immunotherapy involves the targeted immune-based strategies that unleash the patient's immune system to fight cancer. Over the past two decades, with the advances in our understanding of

the regulation of immune responses, immunotherapy has become established as one of the pillars for the cancer treatments.

INTRODUCTION

There has been the rapid growth in the field of tumor immunobiology in recent years as a result of the recent successes for cancer immunotherapies and it became clear that immune cells play sometimes conflicting roles in the tumor microenvironment. However, obtaining of phenotypic information about the various immune cells that play the roles in and around the tumor has been a challenge. Existing methods can either deliver phenotypic information on the homogenous samples (e.g., flow cytometry or PCR) or morphological information for single immunomarkers. We presented here a methodology for delivering quantitative per-cell marker expressions and phenotyping, analogous to that obtained from flow cytometry but from the cells imaged *in situ* in FFPE tissue sections. This methodology combines

with the sequential multi-marker labeling of up to the 6 antigens using the antibodies all of the same species in a single section; automated multispectral imaging (MSI) for removal of the typically problematic FFPE tissue auto fluorescence and for the correct cross-talk between fluorescent channels and automated image analysis that can be quantitative for the per-cell marker expression, determine the cellular phenotype, count these cells separately in that tumor compartment and in the stroma and provide high-resolution images for their distributions. We presented here several examples of the new methodology in the breast, lung, head and neck cancers. Each application will show 6-plex multiplexed staining, per-cell quantitation of each marker and multi-marker cellular phenotyping from the multispectral images of standard clinical biopsy sections as well as methods for the explore the spatial distributions for the phenotyped cells in and around the tumor.

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