Novel immunoscore system development with three-dimensional characterization of optical cleared cancer tissue

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Abstract

Immnoscore provides a reliable estimation of the risk of recurrence through evaluation of immune cells infiltrated or surrounded by the tumor, which has proven to be an effective diagnosis tools to provide a prediction of recurrence risk while used with the common cancer staging method TNM Classification of Malignant Tumors (TNM) scoring. However, the heterogeneity of cancer tissue increase the difficulty of immune environment informatic acquisition by conventional HE and IHC planar images. Thus, analysis of tumor infiltrated immune cells with three-dimensional tissue imaging is considerable a new approach of intra-tumoral immune contexture evaluation.

In this study 10 specimens of breast cancer patients were collected from tissue bank of Chi Mei Medical Center. The specimens were sectioned in 4-μm thin slides and 150-μm thick slides. All thin slides were labeled with anti-CD3 and anti-CD8 antibodies through standard IHC process, which reveal the T cells and cytotoxic T cells in the slides, the labeled slides then digitalized to planar (2D) image by whole slide imaging (WSI) system. In parallel, the thick slides prepared through a process including fluorescence staining, optical tissue clearing to generated optical cleared cancer tissue. These slides imaged by an confocal microscopic system development with integrated resonant scanner and hybrid detector, which the scanning speed is up to 7.7 frame per second (fps) with 1024*1024 digital resolution, to ensure the time consuming of 3D imaging was satisfied for clinical evaluation.

Both 2D and 3D images were recognized and separated into tumor/invasive margin/normal region with an AI-powered algorithm, and quantified the density of of CD3+ and CD8+ cells in the tumor region and in the invasive margin for Immunoscore evaluation. The correlation between 2D/3D images and superiority of 3D imaging methodology facilitate further investigation of intra-tumoral immune contexture of solid tumor, which in turn may contribute to precision diagnosis in support of precision medicine.