Automation of Multiplex Immunohistochemistry

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Multiplex immunohistochemistry (mIHC) is a specialized technique that utilises seven or more antibodies to phenotypically identify different cells in a single tissue section. The Peter Mac's Centre for Advanced Histology and Microscopy (CAHM) routinely optimizes different antibody panels for use in mIHC. Target antigens are visualized using Tyramide Signal Amplification (TSA). Heat induced removal of the primary antibody and secondary Horseradish Peroxidase-conjugated antibody is performed after each round of labeling, leaving the fluorescently conjugated Tyramide covalently bound to tyrosine residues, clustered around the antigen. Removal of primary and secondary antibodies permits the sequential use of antibodies raised in the same species on the same section. Sections labeled by mIHC are imaged with a multispectral camera on the Perkin Elmer Vectra microscope or with a confocal microscope. Using confocal microscopy >7 fluorophores can be spectrally unmixed and fluorophores with a long stokes shift, which is the separation between excitation and emission wavelengths, can be used. The Vectra is currently limited to 7 fluorophores with an emission range between 420 and 720nm but has some advantages over the Confocal microscope: (i) the ability to automatically scan 200 sections, and (ii) the Vectra's powerful analytical software, Inform, which can be trained to automatically segment regions and/or cells of interest.

Two major bottlenecks exist in mIHC; the time taken to manually label each section with 7 different antibodies, and the time taken to perform the subsequent analysis. We have optimized protocols on the BOND RX immunostainer (Leica) to fully automate mIHC, decreasing the time taken to perform 7-color mIHC from 2-3 days to 10 hours. Added advantages of automating mIHC include the ability to label thirty slides simultaneously and excellent reproducibility. Automation also frees up technicians so they can perform other duties. We compared manual mIHC labeling on the bench with automation using sections from multiple different tumour types and different antibody panels. The automated procedure resulted in less background compared to manual labeling and was also gentler on sections. As an open access facility, we now offer high throughput mIHC to both researchers and industry.