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The AstroPath Image Acquisition and Segmentation Workflow

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Multidimensional, spatially resolved analyses of cells from pathology slides are of great diagnostic and prognostic interest. New multispectral, multiplex immunofluorescence microscopy platforms have the potential to facilitate such analyses, and here, we further improve and standardize the image acquisition and cell classification workflow. Studies to date on this emerging technology have typically assessed ~10 operator-dependent high power fields (HPFs) per slide, which represents a fraction of the tissue available for study. Standard cell segmentation and classification algorithms often oversegment larger cells, when they are segmented at the same time as smaller cells. Here we describe our AstroPath imaging platform, which addresses each of these considerations. In our study, slides from formalin-fixed paraffin embedded tissue specimens were stained with an optimized 6-plex multiplex immunofluorescence (mIF) assay. The slides were then scanned at 35 unique wavelengths using a multispectral microscope (Vectra 3.0 or Vectra Polaris) with 20% overlap of HPFs in an operator-independent fashion. An average of 1300 HPFs per slide was required to image the entire tissue, and each microscope scanned between 2 to 3 slides per day with this approach. After the images were captured and organized, overlaps were used to measure, quantify and correct systematics in the imagery (see Eminizer abstract). The central parts of the images were used to create a set of seamless "primary"tiles, similar to the strategy of the Sloan Digital Sky Survey, for a statistically fair pixel coverage of the whole tissue area (see Roskes abstract). Images were then linearly unmixed from the 35 wavelengths to 8 component layers (DAPI, tissue auto-fluorescence, and the 6 added fluorescent dyes) using inForm Cell Analysis©. We then employed a bespoke method for 'multi-pass'classification of cells wherein each marker was segmented and classified separately from the other markers, then merged into a single plane using a unique set of rules and predefined cell hierarchy. We showed that our segmentation and classification method reduced error in over-counting larger cells, e.g. tumor cells, by 25% and increased the specificity and sensitivity in each classification algorithm. Due to the amount of data, each algorithm was run automatically through one of 20 virtual machines housed on a set of servers in the Physics and Astronomy Department. Following the methodology developed during the SDSS project, image data was stored in a well-defined file system structure that facilitated further automatic processing and ingestion into a SQL Server database. Raw data for each slide was 200-300 GBs, which is on par with a full scale (30x) human genome. In summary, we have developed a unique facility and workflow that generates whole slide multispectral imagery with high-fidelity, single cell resolution. Our facility houses five multispectral microscopes (2 Vectra 3.0 and 3 Vectra Polaris) allowing us to collect a petabyte of raw data per year, on scale of the largest sky survey.

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