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Building Seamless Whole Slide Multiplex Images in the AstroPath Project

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The analysis of microscope images is rapidly advancing. Where previously most analyses relied on manual inspection of certain regions of images, advanced quantitative techniques are now being utilized to analyze large sets of entire images at high magnifications. Current multiplex microscopy for cancer imaging in the AstroPath project relies on collecting more than a thousand high resolution images over the tissue on a given microscope slide (see Green abstract). To create a seamless whole slide image over the whole tissue area, a precise registration of individual high-power fields within a single whole slide coordinate system is needed. The mechanical positioning of the slide in the focal plane of the microscope has a small but noticeable jitter, which requires additional compensation before we can assemble the whole slide images. In the AstroPath project, we have designed a strategy that involves collecting overlapping images, similar to the Sloan Digital Sky Survey, enabling the correct segmentation of cells near the edges of the images. This poster describes the AstroPath framework's method for stitching the images together, in which high-power fields are registered using the pixels in the 20% overlap areas. Adjacent high-power fields are pairwise aligned using the cross correlation of their overlapping regions, and a final whole slide registration is performed using a spring-based model to minimize overall pixel shift. The results of the technique are illustrated using sets of archival pathology specimens from patients with melanoma imaged using a Vectra 3.0 microscope. Whole-slide registration with the AstroPath framework reduces the 5th to 95th percentile misalignment error from 3 horizontal pixels and 5 vertical pixels to less than one pixel in both directions, and the cells on the edges of high-power fields, which represent 3-6% of the total number of cells in the image, become more meaningful.

Author: ROSKES, Jeffrey (Johns Hopkins University)

Co-authors: EMINIZER, Margaret (Johns Hopkins University); GREEN, Benjamin (Johns Hopkins University); Mr DOYLE, Joshua (The Johns Hopkins University); Dr WILTON, RIchard (Johns Hopkins University); Prof. TAUBE, Janis (The Johns Hopkins University); SZALAY, Alexander (Johns Hopkins University)

Presenter: ROSKES, Jeffrey (Johns Hopkins University)

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