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# Correcting for Systematics in Multiplex Cancer Imaging in the AstroPath Project

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Historically, analysis of microscope images has relied mainly upon extensive manual review by experienced medical professionals, but recent developments in microscope technology, biological workflows, and data handling capabilities have opened the door to new ways of thinking about microscopy data. The AstroPath group is working to translate a host of "big data" methods familiar in astronomy image analysis to the field of microscopy, with a focus on accelerating the pace of discovery and development by increasing data throughput and accessibility. A main goal is to increase automation in analysis pipelines, which requires a high degree of quantitative precision. To this end, the AstroPath platform includes software methods to characterize and correct systematic effects inherent to microscopy data, and to automatically detect image regions of interest in large datasets. These methods were developed using multiplexed immunofluorescence microscopy data from a cohort of archival pathology specimens from patients with melanoma imaged using an Akoya Biosciences Vectra 3.0 digital microscope. Corrections are made for spatially dependent illumination variation introduced by nonuniformities in the microscope optical paths by averaging over tissue regions in large datasets. Subtle warping effects introduced by microscope objectives and camera lenses are corrected using models from OpenCV optimized by comparing overlapping regions of slides. Otsu's algorithm is adapted to find background thresholds, and tissue is disambiguated from empty background by applying those thresholds and analyzing multiple image layers at once. Blur detection with the Laplacian operator is used to identify regions of images that have been obscured by dust or that depict tissue that is folded over on itself. Images whose exposures are automatically curtailed at the time of collection are used to determine saturation thresholds, and those thresholds are used to identify regions of images that are overbright due to excess stain, the presence of red blood cells, or other exogenous and endogenous artifacts. Overlapping regions of images are compared to identify images that exhibit ambiguous unmixing of their spectral components. Segmenting empty background and artifacts from images and applying the derived corrections is shown to reduce the overall variation in 5th to 95th percentile illumination from 11.2% relative to the mean to 1.2% on average over all image layers.

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