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Immunofluorescence Image Feature Analysis and Clustering Pipeline for Distinguishing Epithelial-Mesenchymal Transition

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Epithelial-Mesenchymal Transition (EMT) is a biological process essential for tissue regeneration and implicated in pathological process such as cancer metastasis, in which epithelial cells transdifferentiate into mesenchymal cells. We devised an image processing pipeline to distinguish between tissues comprised of epithelial and mesenchymal images based on extracted features, using immunofluorescence images which demonstrate differing cellular and tissue structure and biochemical markers. Mammary epithelial cells were incubated with 0ng/mL (control) or 10ng/mL TGF-β1 and cultured for 48 hours. Cells were fixed, stained and imaged for E-cadherin, actin, fibronectin and nuclei via immunofluorescence microscopy. Feature selection was performed on both composites images and different combinations of individual channels using the MATLAB Bag-of-Features tool. Principal Component Analysis (PCA) was performed on the extracted data, and a normalized epithelial and mesenchymal score was defined for each image, based on Euclidean distance from cluster centers. Each image was also rated on a Likert (1-5) scale, by 4 lab members blinded to treatment conditions. PCA produced distinct clusters between epithelial and mesenchymal images. We found that increasing the number of extracted features required more components to explain a given variance. Surprisingly, fewer features produced greater cluster separation and more cohesive clusters. Amongst the analysis of individual channels, imaging of fibronectin yielded the best cluster separation. Lab member ratings agreed closely with epithelial and mesenchymal scores. Overall, the feature extraction and PCA pipeline distinguished epithelial and mesenchymal immunofluorescence images, and future work will focus on characterization of images in intermediate stages of the EMT process.