High Content Image-based Profiling and Analysis of iPSCs
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Introduction: Cell painting is a fluorescence-based assay that can be used to profile cells into morphological features. Compared to traditional methods such as flow cytometry, it requires less experiment level optimization and uses microscopy to capture spatial measurements. Though cell painting assays have been applied to a wide range of cell types, few such studies have involved induced Pluripotent Stem Cells (iPSCs). The aim of this study was to optimize a cell profiling pipeline for use on iPSCs and provide a baseline for their morphology before differentiation. iPSCs have shown promise in regenerative medicine, and are commonly used in disease modeling and drug screenings. Better quality control of iPSCs is necessary to improve research reproducibility, and the assay produced could be used to identify the standard variation and distribution of iPSC features. It could also be used to identify the impact of different treatments on iPSCs. These comparisons allow us to draw more accurate conclusions about iPSC properties and production protocols.

Materials and Methods: A small-molecule differentiation pipeline was followed to stain cells within a 96 well plate. Six fluorescent dyes were used to stain five channels of images. The treatments investigated included a control, DMSO vehicle control (1:1000 v/v), ROCK inhibitor (10 µM), and CHIR (6 µM), IWP (12 µM). A large set of images were acquired using an ImageXpress XLS microscope and converted into a feature set using CellProfiler. Where possible, pipeline modules were configured based on previous research. Other module specifications were identified through manual measurement and segmentation checks. Empty images were flagged to prevent false object identification due to high background noise. Feature selection and dimensionality reduction methods were used to identify feature sets that best encompassed the profiles. Profile similarity across the five conditions was measured and compared.

Results and Discussion: Figure 1. demonstrates the pipeline's ability to correctly segment objects (cells, cytoplasm, nuclei). The pipeline extracts approximately 1400 features for each of these objects, as well as separate image quality measurements. Extracted object features are calculated at a single-cell level, and relate to texture, shape, intensity, and microenvironment. Metadata columns provide information such as image quality flags, well sites, as well as the chemical treatment applied to each well. Image quality features such as saturation and focus scores were exported to a separate dataset, and provide valuable information for image-level quality control. Principal Component Analysis (PCA) was applied and optimized to best differentiate between treatments.

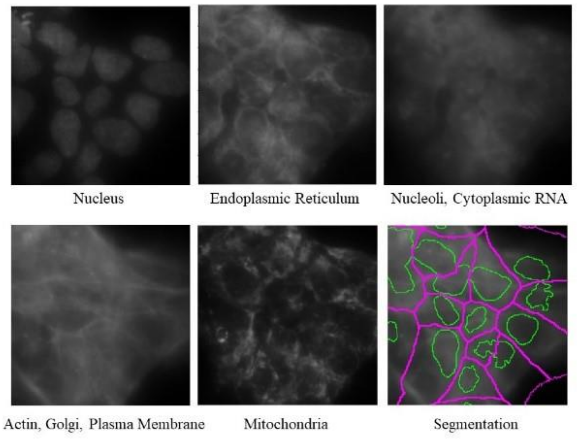

Figure 1. Sample images from a well treated with DMSO, as imaged on an ImageXpress XLS microscope. The five channels and eight organelles examined are shown in the first five images, whereas the last image illustrates the overlaid segmentation of the identified nuclei and cell bodies.

Conclusions: The aim of this study was to develop an assay to profile iPSCs subjected to different chemical perturbations. The pipeline produced allows rapid and inexpensive profiling of iPSCs, and the extracted datasets can provide important information about their morphology. In the future, a wider range of methods should be investigated to ensure assay quality. A larger image set would allow better identification of correlations across conditions. Additionally, there is a demand for new cell painting methods to be continuously developed and optimized.

Acknowledgments: I would like to thank my PI Dr. Zachary Laksman, Mishal Ashraf, and the support team from Compute Canada, for all of the guidance, support, and encouragement they provided. Thank you as well to Lisa Lim, BarRun Kim, and Marvin Gunawan for their help with producing and imaging the iPSCs. Work by this author is supported by the CBR-SBME grant, as well as the Heart Lung Innovation Center.