Deep Learning Based Characterization of Mesenchymal Stem Cells:
Imaging Flow Cytometry-Mediated Detection of Ki67 Expression

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Statement of Purpose: Developments in deep learning have yielded unprecedented technological advancements, and they are improving interpretations of large-scale scientific data. In some applications, the deep learning approach has matched or exceeded human level performance. For instance, there have been great strides in advancing neural networks' prowess in image analysis. Previously, research on analyzing cell images was centered on measuring features that are relevant to a specific phenotype. Now, convolutional neural networks (CNNs) reduce the workflow allowing for whole images to be input directly into a CNN.

In this work, we analyze human bone marrow mesenchymal stem cells (MSCs) for Ki67 expression by training a CNN with pairs of brightfield and fluorescence pictures obtained from an imaging flow cytometer or from monolayer (Figure 1). Significantly, live cells can be analyzed, enabling downstream use in regenerative medicine. This approach would surpass current quality control methods that require greater time and cost, and are terminal to cells. Since there exists a potency-deficient subpopulation in aged MSCs, this technique would be especially impactful by enriching for the most suitable cells for therapy.

Methods: MSCs (10-year-old male donor) were cultured under standard conditions, stained for Ki67, and imaged with an AMNIS Imagestream^X MarkII. Prior to custom CNN processing, the images (1,164 for both positive and negative conditions distinguished by visual inspection) were resized to uniform dimensions (128 x 128 pixels). The CNN architecture consisted of three layers of convolution and max pooling and was terminated by a fully connected layer. The CNN processed the images until additional training epochs did not improve accuracy.

Additional analysis was performed on monolayers of MSCs (22-year-old male donor). After staining for Ki67, Hoechst was added to identify nuclei for cell segmentation. A custom MATLAB script cropped cells centered on the nucleus (128 x 128), and the threshold for positive and negative cells was based on total fluorescence brightness.

Results: Training yielded approximately 96% accuracy in characterizing MSCs from flow for Ki67 after approximately 75 epochs of training with a batch size of 32. MSCs imaged in a monolayer yielded lower accuracy of approximately 74%.

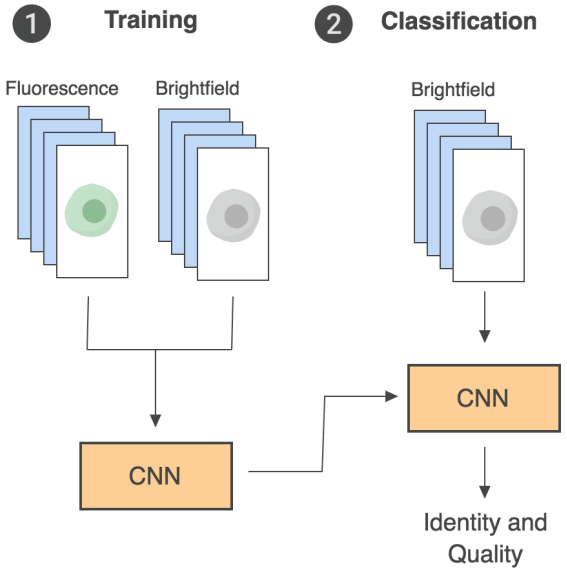

Figure 1. Training and classification workflow. 1) Pairs of fluorescence and brightfield images of cells are used to train a CNN. 2) The trained CNN processes unseen brightfield cell images for their identity and quality.

Conclusions: Our data supports the hypothesis that a CNN can be used to classify cells from brightfield images for Ki67 expression, and more testing is warranted to confirm and expand upon these initial results. Specifically, it should be investigated whether a CNN trained with images of cells in monolayer can result in equivalent classification accuracy relative to training with images from flow cytometry. Future directions include testing with additional markers and integrating this technology into a system capable of sorting for enrichment. This technique may also have broad applicability to other cell types and their phenotypes. Ultimately, it is envisioned that this work will enable viable cell sorting and characterization for cell enrichment and regenerative medicine.