# SVSIS

# Robust morphology-based classification of cells following label-free cell-by-cell segmentation using convolutional neural networks

ව 1.6

<u>–</u> 1.4

Test

Image

13

Test

Image

2.2

50

Test

Test

16

16

Gillian F. Lovell<sup>1</sup>, Christoffer Edlund<sup>2</sup>, Rickard Sjöegren<sup>2</sup>, Daniel A. Porto<sup>3</sup>, Nevine Holtz<sup>3</sup>, Nicola Bevan<sup>1</sup>, Jasmine Trigg<sup>1</sup>, Johan Trygg<sup>2</sup>, Timothy Dale<sup>1</sup>, Timothy R. Jackson<sup>1\*</sup> 1 Sartorius, Royston, SG8 5WY, UK. 2. Sartorius, Umeå. 3 Sartorius, Ann Arbor, MI \* Corresponding author: <u>Timothy.Jackson@sartorius.com</u>.

### Introduction

- Light microscopy is a cost-effective, noninvasive, accessible modality for high-throughput live-cell imaging.
- Accurate segmentation of individual cells enables exploration of complex biological guestions, particularly related to morphological change, but require sophisticated algorithms such as convolutional neural networks (CNNs).
- Many deep learning studies have limited amounts of quality training data.
- We previously reported on LIVECell, an opensource, high-guality, manually annotated and expert-validated dataset, comprising over 1.6 million annotated cells of 8 highly diverse cell types from initial seeding to full confluence, acquired on the Incucyte®.
- With minimal additional data, we fine-tune one of our publicly available LIVECell-trained models to enable quantitative analysis of complex morphological change associated with two applications, cell viability and differentiation.

### Incucyte® Live-cell imaging and analysis systems



**High-throughput Image Acquisition** Ideal for Deep Learning Applications The Incucyte® generates thousands of highquality HD phase images from a single experiment. Fluorescence imaging capabilities also facilitate data generation for validation purposes.

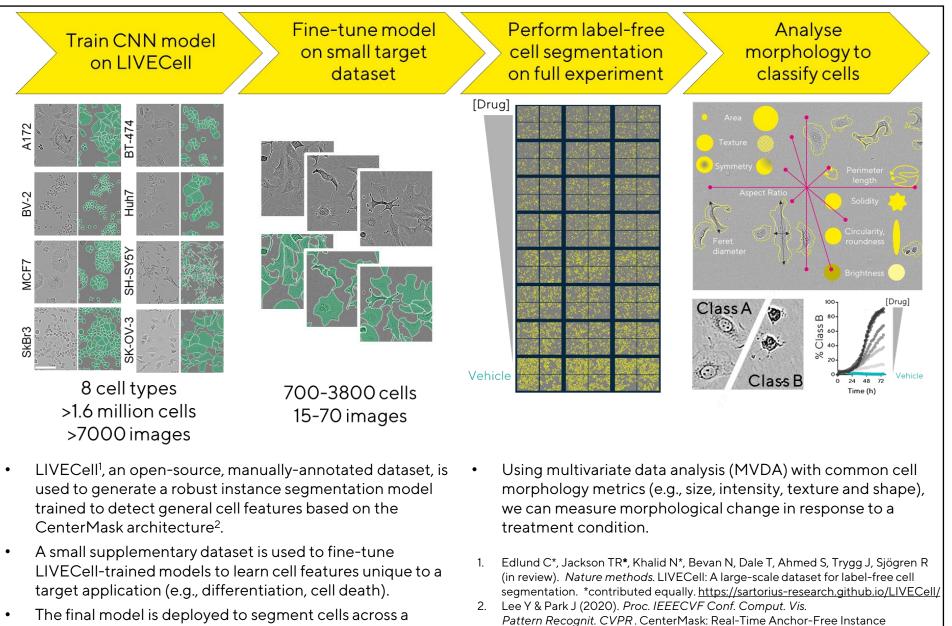
complete experiment.

Integrated Software Integrated software enables individual cells to be segmented, and analysis of single metrics (area, fluorescence within the cell).

#### Advanced Data Analytics

Incucyte® Advanced Label-free **Classification Module enables** quantification based on cell morphology; convolutional neural networks (CNNs) can be used for improved cell segmentation.

### LIVECell enables morphological analysis of cells with minimal additional annotated data

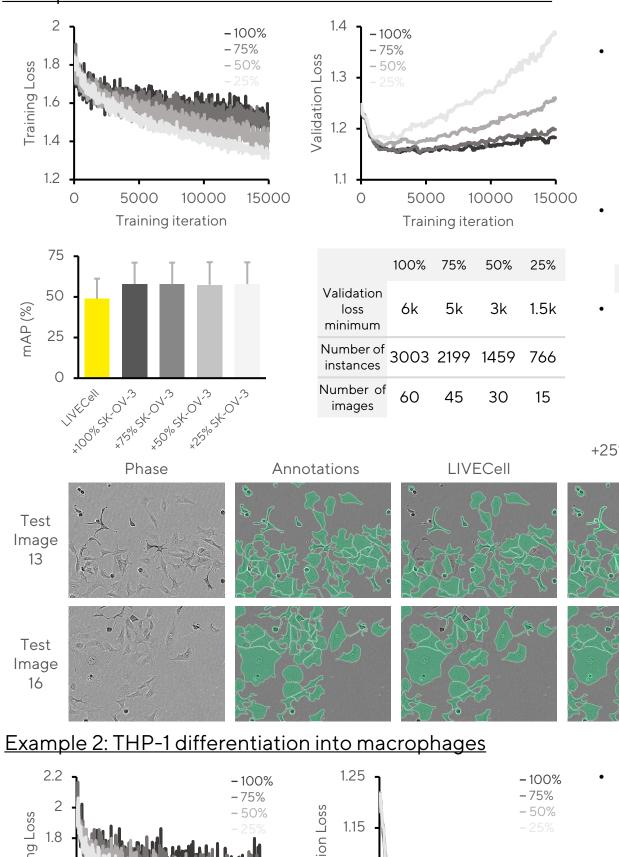


Segmentation, 13906-13915

### Application-specific CNN model fine-tuning

Improving the cell segmentation accuracy for specific applications requires minimal additional data to fine-tune LIVECell-trained CNN models

Example 1: Treatment-induced cell death in SK-OV-3 cells



1.05

09

Validation

loss

minimum

instances

Number of

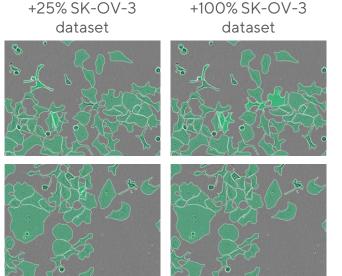
8k

68

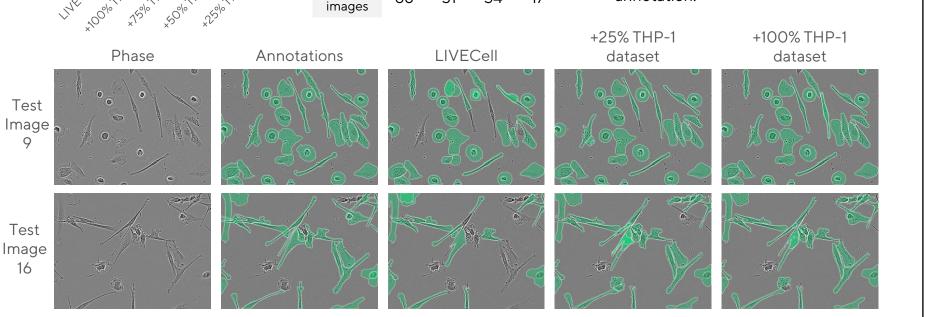
5000 10000 15000

Training iteration

- 84 HD phase images of SK-OV-3 cells treated with the cytotoxic agent vinblastine were manually annotated
- To explore the data requirements to finetune a LIVECell-trained model, a further 15K training iterations were performed using training sets of various sizes, using between 25-100% of the 60 available training images. 8 images were reserved for validation, 16 were reserved for model evaluation.
- Loss, calculated at each iteration for the training dataset, and every 20 iterations for the validation dataset, is defined as:
- $Total \ Loss = L_{class} + L_{center} + L_{bhox} + L_{mask}$
- Using the model checkpoint nearest the minimum validation loss, we find that the mean average precision (mAP) was not largely impacted by the training dataset size. Using just 15 images resulted in a 9point increase in mAP (48.9 vs 57.9%)



- 95 HD phase images from a THP-1 macrophage differentiation plate were manually annotated. 8 images were reserved for validation, 19 were reserved for model evaluation. Training set size was varied as above (see table on left).
- We find the mAP on the test set to have increased nearly 20 percentage points with even the smallest training set size (38.9% vs. 58.2%)
- We note a moderate decrease in mAP relative to training dataset size, when using 100% of available training images (mAP = 60.4%) compared to using 25% (mAP = 58.2%)
- Inspection of segmentation masks on the test dataset reveals robust masking compared to the ground truth annotation.



5000 10000 15000

4k

34

17

Training iteration

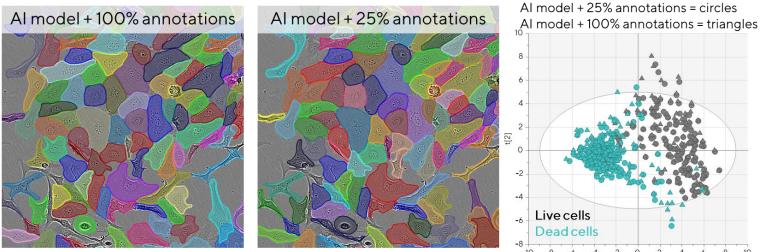
6k

51

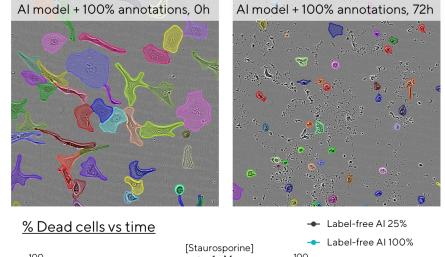
Number of 3746 2753 1837 866

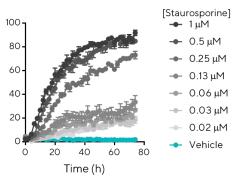
100% 75% 50% 25%

### Segmentation data provides biological insight

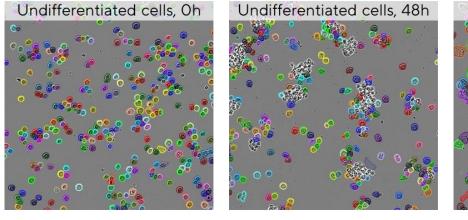


- separately from dead cells (teal)

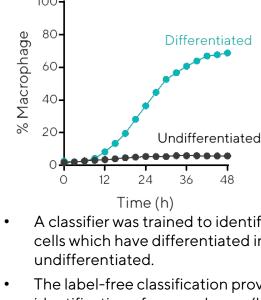




#### Morphological data enables label-free quantification of monocyte differentiation



Label-free quantification of macrophages:



- the requirement for fluorescence reagents and imaging.

## Simplifying Progress

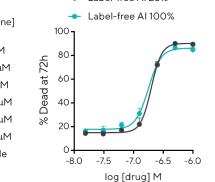
#### Morphological information on cells can be derived from the cell segmentations

- Cell morphology is typically described qualitatively.
- lt can alter in response to biological events including cell death or differentiation.
- Segmentation vields morphology data e.g. area, roundness, texture.

Images show SK-OV-3 cells qualitatively similar segmentation performance using the model with 100% (left, +100%) or 25% additional annotated images (right, +25%). 25 metrics describing aspects of the morphology of every cell was extracted. Principal component analysis (PCA) of morphology metrics shows that live cells (grey) have a wide distribution and cluster

Objects segmented with the +25% model (circles) also lie close to the objects segmented with the +100% model (triangles) indicating that the information extracted from both models is comparable.

#### Cell viability can be quantified from cell morphology data using non-perturbing live cell imaging

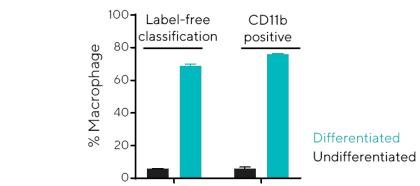


SK-OV-3 cells were treated with the staurosporine (20  $nM - 1\mu M$ ) to induce cell death. Cells were placed in an Incucyte® and Phase HD images were acquired every 2h for 3 days (images shown at 0h and 72h treatment).

- Cells were segmented and the morphological data extracted. Using a custom version of the Incucyte ${
  m extbf{B}}$ Advanced Label-free Classification Module, a classifier was trained to identify cells as live or dead.
- This classifier was applied to all images and the % dead cells per image was quantified (timecourse, left) to demonstrate time- and concentration-dependent increases in cell death.
- Concentration response curve (right) shows the % dead cells at 72h across compound concentrations. The +25% and +100% models yielded similar efficacy values  $(plC_{50} = 6.7).$
- This demonstrates that robust AI-based segmentation enables highly accurate and label-free quantification of dead cells.

Differentiated cells, 48h

Comparison to standard fluorescence method



- THP-1 monocyte cells were treated to induce differentiation.
- Differentiation causes the cells to alter morphology over time from small rounded objects (left hand image, Oh) assuming a larger, elongated and adherent phenotype typical of macrophages (right hand image).
- Cells were segmented using the model +100% available THP-1 images.
- The segmentation excludes clusters enabling the analysis to be performed on single cells only.

A classifier was trained to identify monocytes vs macrophages. Timecourse (left) shows that in the presence of PMA the % cells which have differentiated into macrophages increases over the 48h period; in the absence of PMA cells remain

The label-free classification provides comparable results to standard immunocytochemistry using CD11b as a marker for identification of macrophages (bar graph, right).

These data show that changing cell morphology can be quantified and specific cells identified using Phase HD images without