

Classification of cell morphology using machine learning and label-free live-cell imaging

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Introduction

- Cell morphology is a strong indicator of cell viability and phenotype. We have developed a workflow for robust label-free classification of user-identified cell morphologies based on segmented Phase HD images
- We demonstrate two applications of this method for quantification of % dead cells in a cytotoxicity assay and of % macrophages in a differentiation assay
- Incucyte® Live-cell Analysis Systems are ideal for long-term morphological analysis as they continuously acquire images from within an incubator without perturbing the cells
- Integrated software automatically segments individual cells after each image acquisition and users can perform label-free classification based on total morphology (Incucyte® Advanced Label-free Classification Module, available with software v2021B)
- A convolutional neural network has been trained using Incucyte® images to segment individual cells resulting in more accurate morphological readouts (integrated solution available in a future software release).

Incucyte® Live-cell analysis systems

Image Acquisition
Incucyte® Live-cell Analysis Systems are a uniquely powerful technology for quantifying cell morphology. Phase HD images of live cells are acquired from within an incubator without perturbation.

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 shape; artificial neural networks can be used for improved cell segmentation.

Integrated software enables simple and accurate classification based on morphology

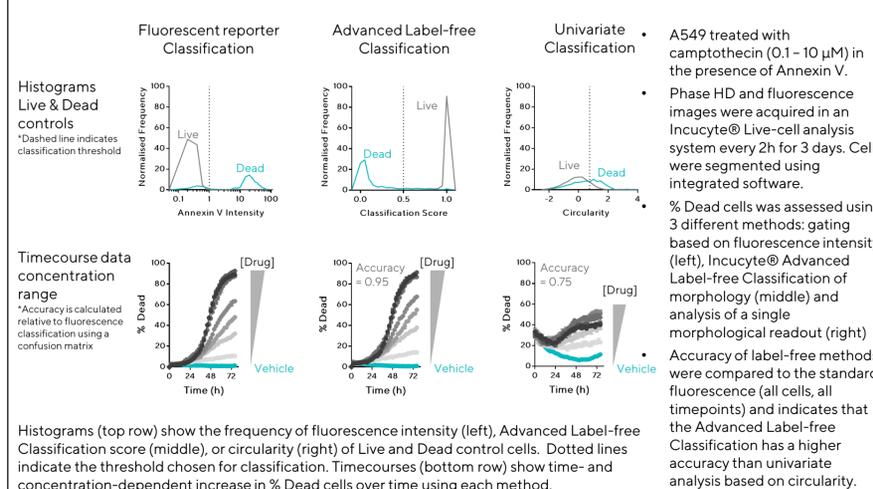
Live-cell imaging → **Cell segmentation** → **Integrated total morphology analysis** → **Train classifier using control images** → **Cell classification**

Morphological metrics analyzed include: Area, Texture, Symmetry, Perimeter length, Aspect Ratio, Solidity, Circularity, roundness, Feret diameter, and Brightness.

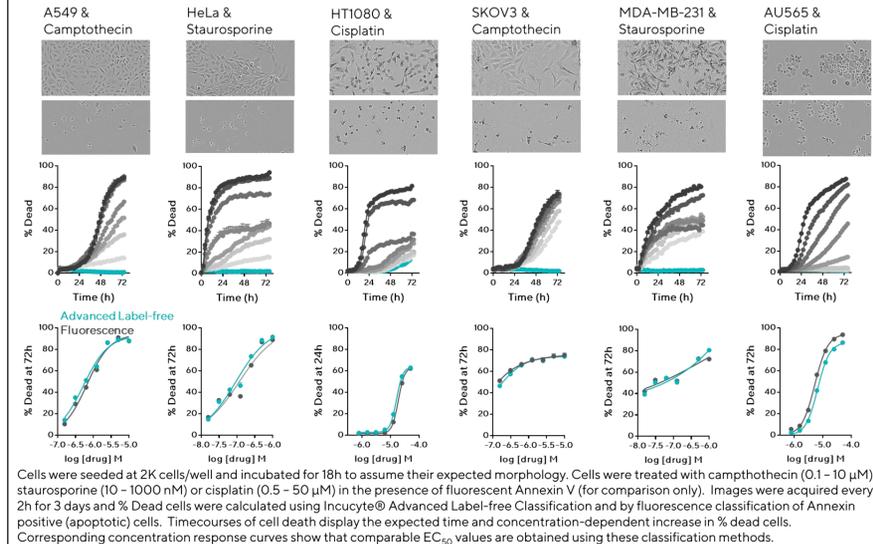
- Phase images of cells are acquired without perturbation within an incubator for the entire timecourse of an assay.
- Cells are segmented in real time using integrated Incucyte® software.
- Control wells are set up to generate images of 2 classes of different morphologies (e.g. Live vs Dead).
- Complex morphological data describing a large number of variables (shape, texture, brightness) is distilled to a single axis using multivariate analysis (MVA)
- An MVA regression model is trained to identify the 2 control classes using the segmented images.
- The model can then be deployed on all other images generating a numerical score for every cell from 0 to 1.
- (Optional) For comparison, standard fluorescence classification can be performed.
- Setting a score threshold (e.g. 0.5) classifies cells as Class A or Class B (e.g. Live or Dead).
- Classification masks identify the class of each cell in the image.
- % Dead cells can be calculated from Phase HD images without the use of fluorescent reagents.

Label-free Live/Dead Assay

Incucyte® Advanced Label-free Classification of dead cells is comparable to the use of fluorescent cell health reagents and has higher accuracy than other label-free methods



Incucyte® Advanced Label-free Classification is applicable to a wide range of cell morphologies and treatment conditions



Label-free Differentiation Assay classifies macrophages based on morphology

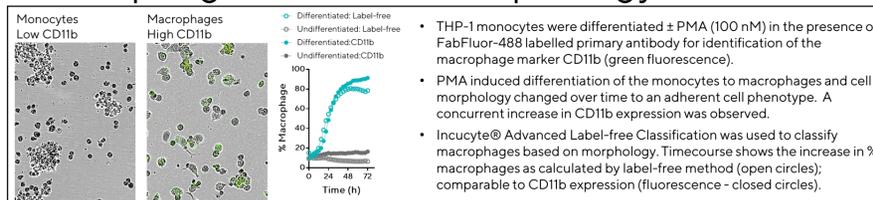
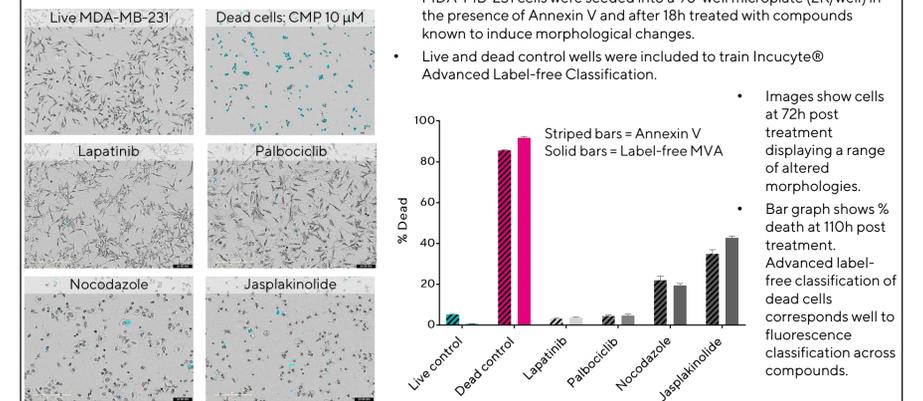
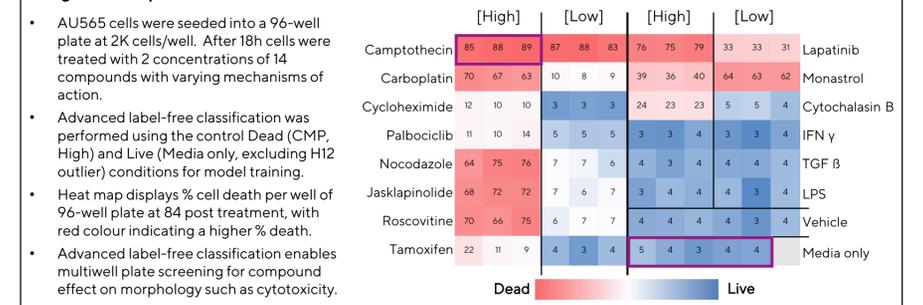


Image-based morphology screening

Incucyte® Advanced Label-free Classification robustly identifies dead cells in the presence of other morphologies



Label-free phenotypic screening based on cell morphology indicates % cell death induced by a range of compounds with different mechanisms of action



AI-based cell segmentation increases accuracy of morphology data and enables Incucyte® Advanced Label-free Classification

An AI-based segmentation approach enables accurate delineation of cell boundaries of both healthy (top row) and treated (Tamoxifen, 20 µM, bottom row) MCF7 cells. Segmented cells at 0, 48 and 72h indicate the approach is highly adaptable to changing morphologies. Timecourse of cell count indicates that healthy cells proliferate while treatment with chemotherapeutic agents suppresses growth. Advanced label-free classification of cell morphology has been used to identify dead cells and quantify the cytotoxic effect of chemotherapeutics commonly used to treat breast cancers.

