SARDRICS

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 perturbance.

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



- 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).
- An MVA regression model is trained to identify the 2
- control classes using the segmented images.
- The model can then be deployed be on all other images generating a numerical score for every cell from 0 to 1.
- (Optional) For comparison, standard fluorescence classification can be performed.
- 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.

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

Histograms Live & Dead controls Dashed line indicates classification threshold

Timecourse data concentration range Accuracy is calculated elative to fluorescence classification using a confusion matrix

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









Label-free Live/Dead Assay



Histograms (top row) show the frequency of fluorescence intensity (left), Advanced Label-free Classification score (middle), or circularity (right) of Live and Dead control cells. Dotted lines indicate the threshold chosen for classification. Timecourses (bottom row) show time- and concentration-dependent increase in % Dead cells over time using each method.

camptothecin $(0.1 - 10 \mu M)$ in the presence of Annexin V. Phase HD and fluorescence images were acquired in an Incucyte® Live-cell analysis system every 2h for 3 days. Cells were segmented using integrated software. % Dead cells was assessed using 3 different methods: gating

based on fluorescence intensity (left), Incucyte® Advanced Label-free Classification of morphology (middle) and analysis of a single morphological readout (right) Accuracy of label-free methods were compared to the standard fluorescence (all cells, all timepoints) and indicates that the Advanced Label-free Classification has a higher accuracy than univariate analysis based on circularity.

Cells were seeded at 2K cells/well and incubated for 18h to assume their expected morphology. Cells were treated with campthothecin (0.1 – 10 μ M), staurosporine (10 - 1000 nM) or cisplatin (0.5 - 50 μ M) in the presence of fluorescent Annexin V (for comparison only). Images were acquired every 2h for 3 days and % Dead cells were calculated using Incucyte® Advanced Label-free Classification and by fluorescence classification of Annexin positive (apoptotic) cells. Timecourses of cell death display the expected time and concentration-dependent increase in % dead cells. Corresponding concentration response curves show that comparable EC_{50} values are obtained using these classification methods.

Label-free Differentiation Assay classifies macrophages based on morphology

- THP-1 monocytes were differentiated ± PMA (100 nM) in the presence of FabFluor-488 labelled primary antibody for identification of the macrophage marker CD11b (green fluorescence).
- PMA induced differentiation of the monocytes to macrophages and cell morphology changed over time to an adherent cell phenotype. A concurrent increase in CD11b expression was observed.
- Incucyte® Advanced Label-free Classification was used to classify macrophages based on morphology. Timecourse shows the increase in % macrophages as calculated by label-free method (open circles); comparable to CD11b expression (fluorescence - closed circles).

of other morphologies



Incucyte® Advanced Label-free Classification robustly identifies dead cells in the presence MDA-MB-231 cells were seeded into a 96-well microplate (2K/well) in Dead cells: CMP 10 µM the presence of Annexin V and after 18h treated with compounds Live MDA-MB-231 known to induce morphological changes. au, 6' Live and dead control wells were included to train Incucyte® Advanced Label-free Classification Images show cells at 72h post treatment Striped bars = Annexin V Solid bars = Label-free MVA displaying a range Palbociclib of altered morphologies Bar graph shows % death at 110h post treatment. Advanced labelfree classification o Jasplakinolide dead cells corresponds well to fluorescence classification across compounds. Label-free phenotypic screening based on cell morphology indicates % cell death induced by a range of compounds with different mechanisms of action [Low] [High] [Low] [High] AU565 cells were seeded into a 96-well plate at 2K cells/well. After 18h cells were Camptothecin 85 88 89 87 88 83 76 75 79 33 33 ³¹ Lapatinib treated with 2 concentrations of 14 64 63 62 Monastrol compounds with varying mechanisms of Carboplatin 70 67 63 36 action. Cycloheximide¹²¹⁰ 3 3 24 23 23 ⁵ ⁵ ⁴ Cytochalasin B Advanced label-free classification was performed using the control Dead (CMP, 3 3 4 3 3 4 IFN v Palbociclib¹¹¹⁰ 5 5 5 High) and Live (Media only, excluding H12 4 3 4 4 4 4 TGF B Nocodazole 64 75

- outlier) conditions for model training.
- Heat map displays % cell death per well of 96-well plate at 84 post treatment, with red colour indicating a higher % death.
- Advanced label-free classification enables multiwell plate screening for compound effect on morphology such as cytotoxicity.

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

An Al-based segmentation approach enables accurate delineation of cell boundaries of both healthy (top tow) 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.



Simplifying Progress

Image-based morphology screening

Jasklapinolide 68 72 4 4 4 3 4 LPS 4 4 4 4 3 4 Vehicle Roscovitine 70 66 Tamoxifen 22 4 3 4 4

Dead

Live

Media only