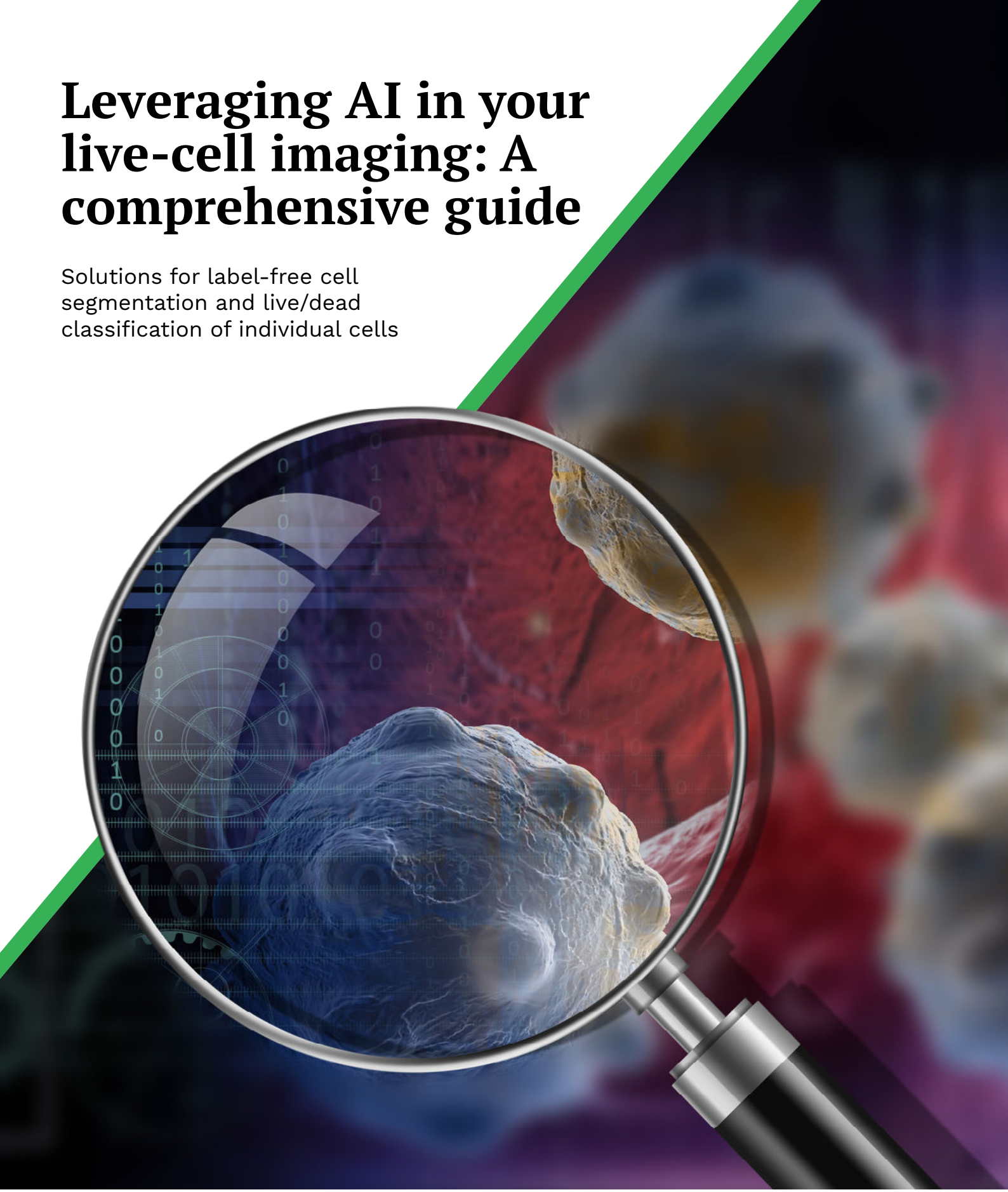
Leveraging AI in your live-cell imaging: A comprehensive guide

Solutions for label-free cell segmentation and live/dead classification of individual cells



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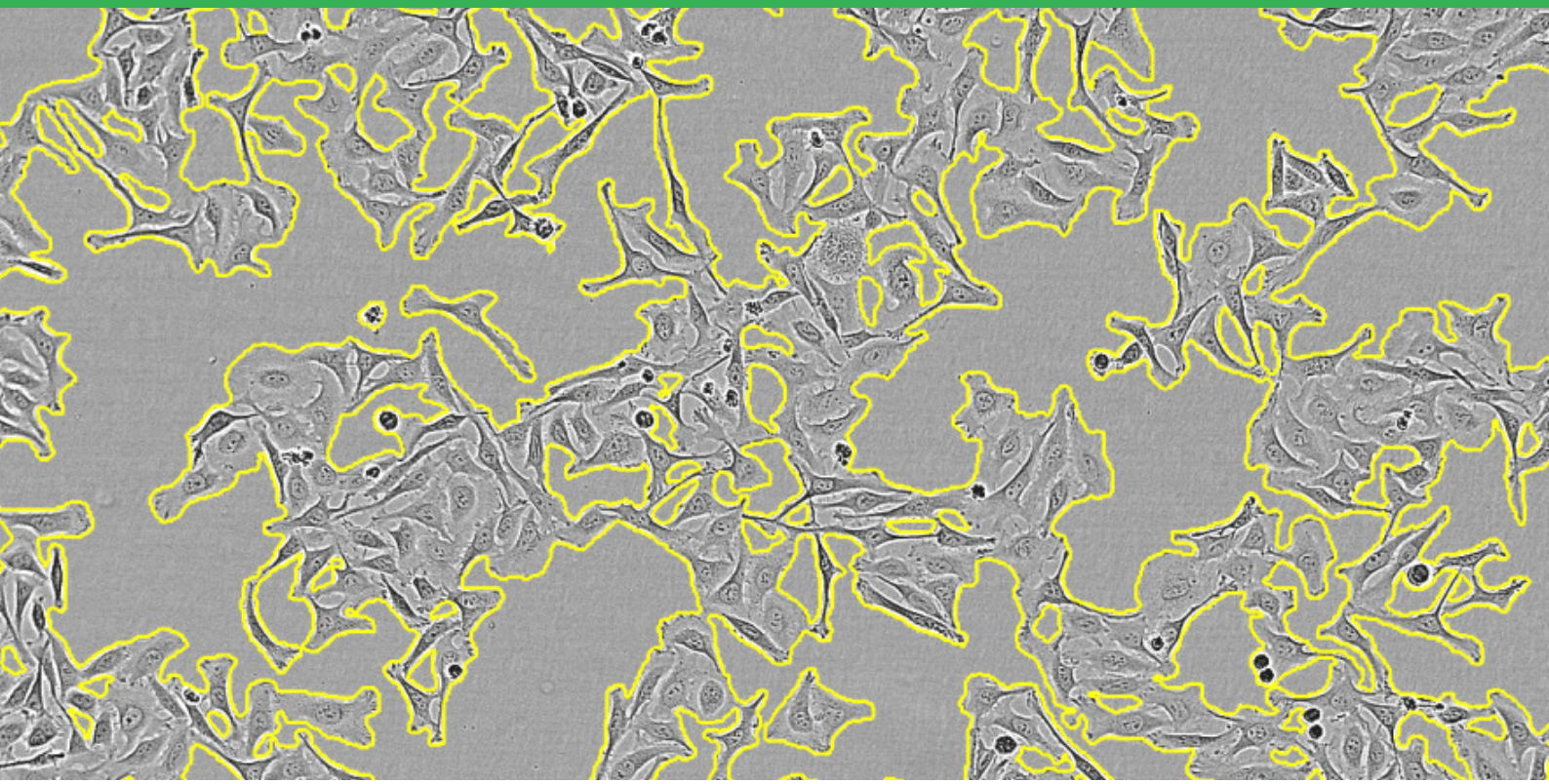

Introduction

Live-cell imaging is a powerful technique used to observe and analyze the behavior of cells in real-time. As cellular models are progressively increasing in complexity this has driven the need for label-free and non-perturbing approaches to accurately quantify dynamic cellular processes. The incorporation of artificial intelligence (AI) and advanced machine learning (ML) into image analysis workflows is now streamlining this process and enabling robust, user-friendly quantification of a wide range of cellular models.

Live-cell imaging provides an ideal platform to study multifaceted biological paradigms in drug discovery. Biological models are becoming increasingly complex, using more physiological relevant and precious cell types, and this highlights the importance of label-free analysis methods that are non-perturbing and eliminate any artifacts introduced through using fluorescent labels.

A new generation of label-free image analysis tools, including AI and ML, are emerging to overcome challenges associated with the handling and processing of complex biological images and the interpretation of

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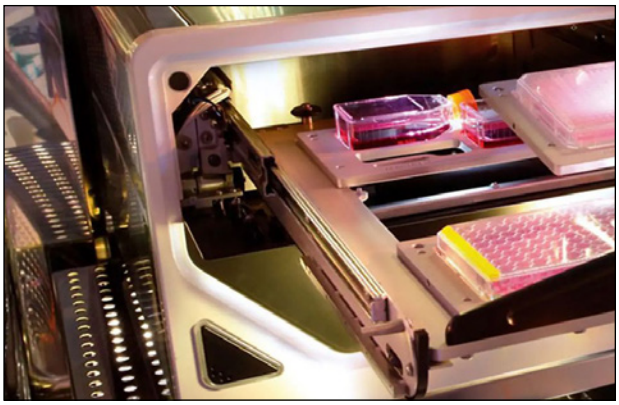
increasingly large amounts of data. Neural networks can be trained for specific tasks, such as label-free cell segmentation, and provide more accurate results than the use of traditional algorithms. These AI-tools can detect cells, identify cellular structures, and extract quantitative measurements from complex image datasets in a highly objective and reproducible manner. The development of advanced machine learning approaches has facilitated data interpretation to gain deeper insights from biological models. For example, cell morphology reveals valuable insight into cell health, phenotype, and behavior, with heterogeneity existing in even the simplest of cell models. Traditionally, cell morphology is assessed qualitatively or quantitatively using univariate shape metrics such as cell area. However, the use of higher-order analytical tools, such as multi-variate analysis (MVA), enables multiple aspects of cell morphology to be quantified simultaneously, providing objective and meaningful insights into cell viability and behavior.

Through the combination of these advanced image analysis methods with specialized live-cell imaging systems researchers can overcome the challenges of traditional workflows, simplify image analysis, and extract meaningful information from their images. Incucyte® Live-Cell Analysis Systems, developed by Sartorius, incorporate a microscope that resides inside a standard tissue culture incubator and acquires high-definition phase-contrast and fluorescence images from underneath cell culture vessels in a physiologically relevant manner. This non-perturbing approach enables visualization and image-based quantification of cellular behaviors across the entire assay time-course, from minutes to months, and is amenable to a wide range of applications such as proliferation, migration, and protein trafficking. Incucyte® Live-Cell Analysis Systems offer a suite of powerful computational tools which utilize integrated AI-driven or advanced analytical approaches. Purpose-built analyses enable high-throughput solutions for robust label-free image analysis with streamlined workflows. These automated tools require minimal user input and ensure these technological advancements are accessible to all biologists.



Incucyte® Live-Cell Analysis Systems enable live-cell analysis inside an incubator

The Incucyte® AI Confluence Analysis, incorporated into the base analysis of every Incucyte® is driven by a pre-trained neural network and allows users to reliably monitor cell proliferation across a wide range of cell types in a non-perturbing manner with minimal user input. The Incucyte® AI Cell Health Analysis is driven by two pre-trained neural networks, which enable the segmentation of individual cells and simultaneous classification of cell viability, allowing researchers to conduct label-free real-time measurements of cell growth and cytotoxicity.

The Incucyte® Advanced Label-Free Classification Software Module uses sophisticated multivariate analysis to kinetically monitor two user-defined populations based on multiple aspects of cell shape. This simplified workflow can be applied to biological models that undergo morphological changes including live/dead and differentiation assays.

This article collection provides an overview of recent label-free image analysis developments and focuses on how AI-driven image analysis is transforming live-cell imaging. In this eBook, we highlight how intuitive label-free analysis simplifies quantification of complex biological behavior and exemplify its application to the drug discovery process through providing high-throughput physiologically relevant insights into cell health and compound efficacy.

Quantifying cell morphology

Cell morphology encodes essential information on many underlying biological processes and is one of the strongest indicators of cell health. Understanding cell morphology can be complicated due to cells changing their morphology because of crowding, cell death, etc. In this application note, discover how advanced analysis tools can be combined to perform multivariate analysis in one workflow to provide actionable insights into cell morphology. This poster further describes a workflow for the label-free classification of cell morphologies based on segmented Phase HD images.

Alongside AI-driven confluence measurements Incucyte® Live-Cell Analysis provides real-time information on cell growth and proliferation. This poster describes a workflow for robust label-free classification utilizing the Incucyte® Advanced Label-Free Classification Software Module.

Label-free cell segmentation using AI

Cell segmentation algorithms are trained using large, well-annotated imaging datasets. Most of the available algorithms, however, are designed for fluorescence-based cell imaging. In this application note, learn how LIVECell (label-free *in vitro* image examples of cells) was created to help address this gap. LIVECell is a large, high-quality, expertly annotated dataset of phase-contrast cell images that can be used for training cell segmentation algorithms for biologically relevant cell imaging experiments.

This poster describes how one of the publicly available LIVECell-trained models was fine-tuned to enable quantitative analysis of complex morphological change associated with cell viability and differentiation.

AI-driven image analysis for cytotoxicity screening

The increasing use of patient-derived cells has driven the need for non-perturbing and label-free cell measurements. In this poster, explore how AI-driven image analysis is enabling simplified, label-free cytotoxicity screening.

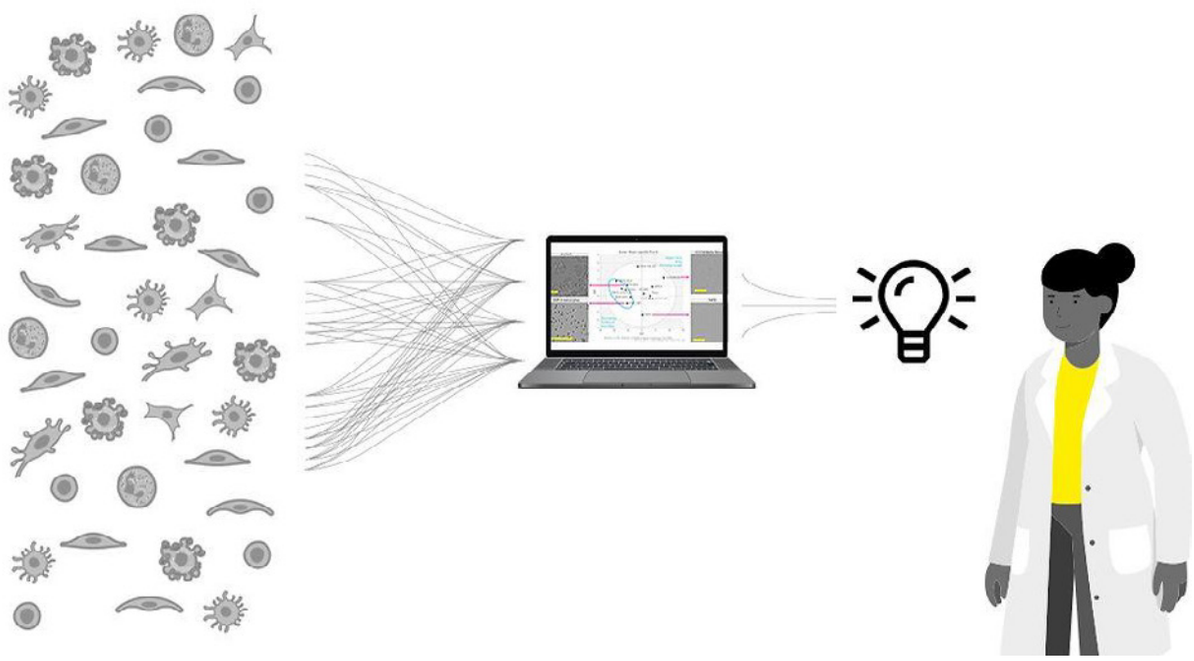
This poster describes a robust *in vitro* assay using the Incucyte® Live-Cell Analysis System and integrated Incucyte® AI Cell Health Analysis Software to assess the cytotoxic effects of clinically relevant chemotherapeutics in established glioblastoma multiform (GBM) cell lines.

Quantification of monocytes using live-cell analysis

Monocytes play critical roles in innate immunity by migrating to inflamed tissue, where they clear micro-organisms and apoptotic cells, repair injured tissues, and recruit other immune cells. This poster describes robust *in vitro* assays for the kinetic evaluation of monocytes and exemplifies how these further our understanding of their biological roles.

How to Go from Cell Image to Insight, with Multivariate Analysis

Biologists spend a lot of time observing cells under the microscope and making qualitative assessments. Do they look right? Are they dividing? What's the differentiation status? These observations inform many important downstream decisions. But the hardest question remains: how can we get quantitative insights from complex cell morphology data?

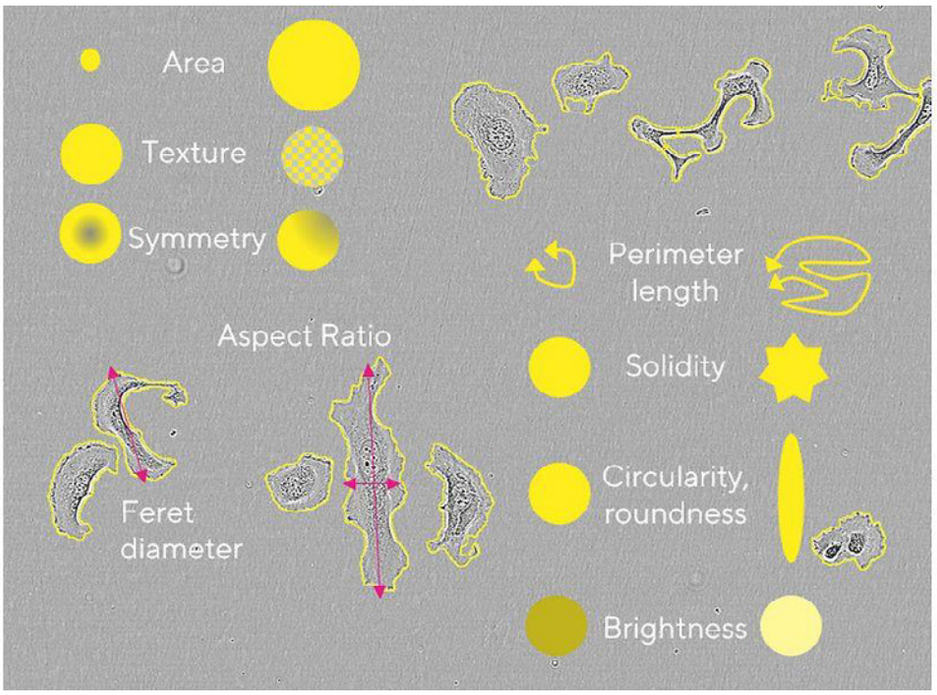


Why Quantify Cell Morphology

Cell morphology is one of the strongest indicators of cell health. But understanding it can be complicated as cells change their morphology for a variety of reasons. Let's say your cells are shrinking. Is it because they are dividing, dying, or simply running out of space?

Quantifying cell morphology over time helps with quality control both to identify cross-contamination of cell types, and to ensure that cell lines maintain consistent properties over multiple rounds of subculture. In this context, cell morphology can change for a variety of reasons:

- Crowding: With increasing confluence, some cells become smaller and more homogeneous in size and shape.
- Environmental/chemical: External triggers can cause blebbing, rounding or other unusual appearances.
- Cell death: Regardless of the initial morphology, dying cells become small, circular, and highly textured.



Advanced Tools Empower Cell Analysis

Developments in cell imaging and computational power have transformed how scientists analyze cells. Unlike traditional microscopy methods that are cumbersome and slow, advanced systems allow direct visualization and quantitative tracking of multiple parameters related to cell health, morphology and function.

One example are the Incucyte® Live-Cell Analysis Systems. Each system incorporates a microscope that sits inside a tissue culture incubator. Without ever moving the cells, you can acquire HD phase and fluorescence images of live cells and automatically segment and analyze individual cells using software tools.

Deriving Insights Using Multivariate Analysis

With today's instrument technologies it's easy to amass a lot of imaging data quickly. The next step is translating complex datasets into meaningful quantitative information that help scientists make decisions.

Software tools are increasingly bridging the gap between multi-dimensional data and actionable insights. For example, metrics relating to cell shape, such as area, texture, roundness, and perimeter length can be analyzed individually (univariate analysis) or using a combination of metrics (multivariate analysis).

Solutions like the SIMCA® software package have tools for multivariate data analysis, which is used to explore relationships between variables, or factors, in complex datasets.

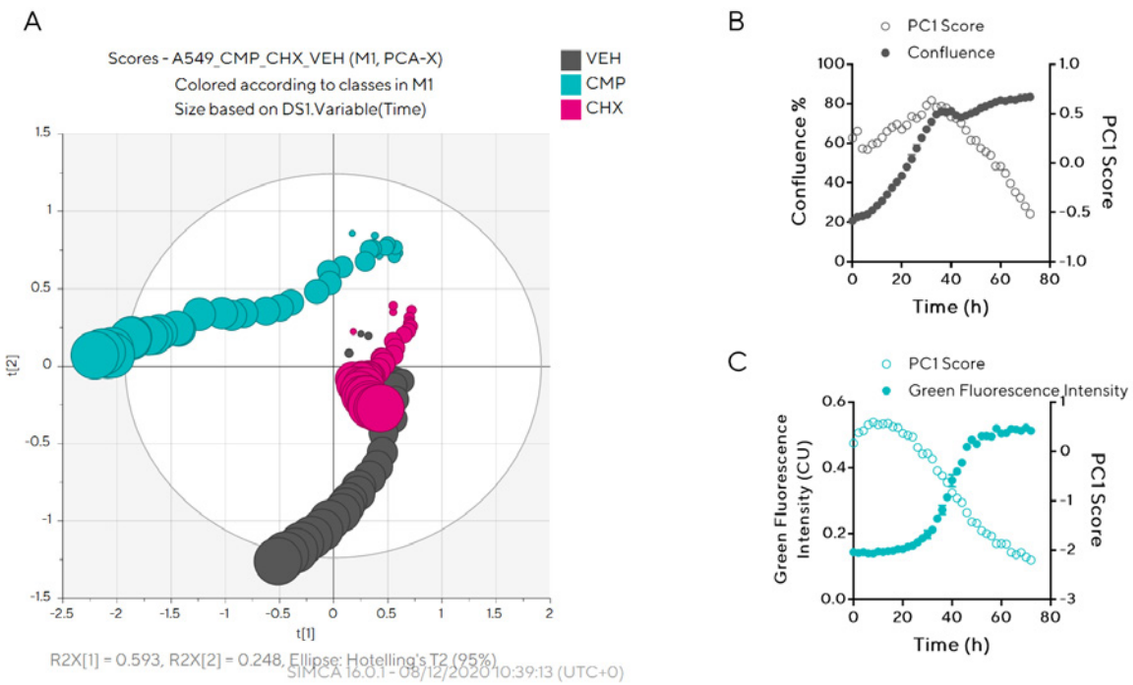
Analyzing Cell Morphology Over Time

This example demonstrates how two such advanced tools can be combined to perform multivariate analysis in one workflow.

Here, cell data was first acquired on an Incucyte® Live-Cell Analysis System and segmented using the integrated software. Next, segmentation metrics were entered into SIMCA® for principal component analysis, which is a type of multivariate analysis, that simplifies high-dimensional data and clusters data based on patterns in an unbiased way.

This analysis represents A549 cells treated with compounds that either arrest cell growth (cycloheximidex), or cause cell death (camptothecin). The PCA plot visualizes the change in morphology over time:

- Arrested cells (pink), which do not increase in confluence, show very little change over time.
- Dying cells (teal) show rapid change and increased activity from pro-death caspase enzymes, as expected.
- Healthy cells (grey), which are actively growing, show more change over the same time period.



Footnote for image: Quantification of morphology in healthy cells (vehicle control, VEH, grey), arrested cells (cycloheximide, 1 µM CHX, pink) and dying cells (camptothecin, 10 µM CMP, teal) using principal component analysis and compared on a PCA score plot (A). Time is indicated by circle size where the smallest circle corresponds to 0h, and largest to 72h. Overlay of vehicle cell confluence and change in PC1 over time (B). Overlay of PC1 and caspase activation (green fluorescence intensity) in CMP-treated cells (C).

The ability to quantify total cell morphology from microscopy images is a huge untapped resource. Combining live-cell imaging with multivariate analysis has created a powerful workflow for analyzing total cell morphology through time.

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Simplifying Progress

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

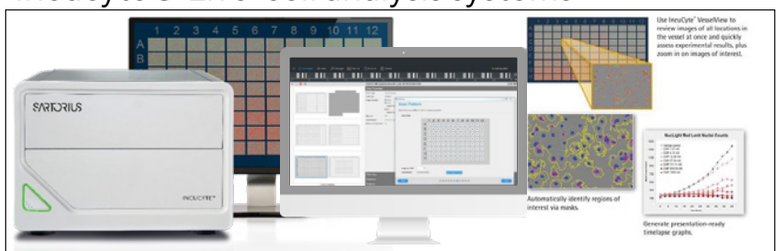
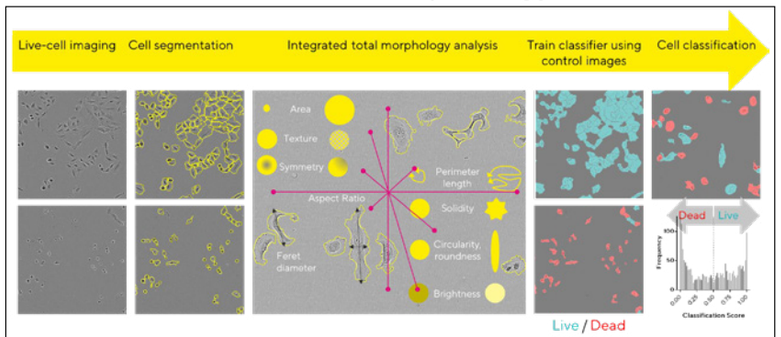


Image Acquisition
Incucyte® Live-cell Analysis Systems are a uniquely powerful technology for quantifying cell morphology. Phase HD images of live cells are acquired from within an incubator without perturbance.

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.

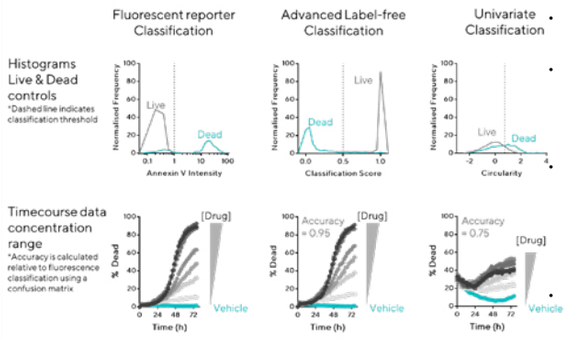
Integrated software enables simple and accurate classification based on morphology



- Phase images of cells are acquired without perturbance 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 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.
- 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

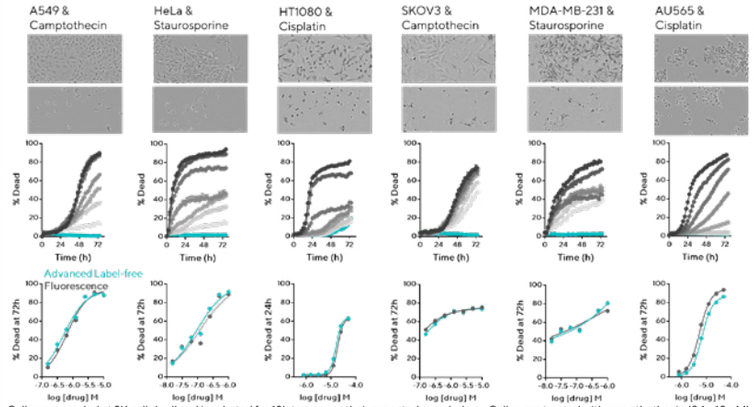
Incucyte® Advanced Label-free Classification of dead cells is comparable to the use of fluorescent cell health reagents and has higher accuracy than other label-free methods



- A549 treated with camptothecin (0.1 – 10 µM) 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.

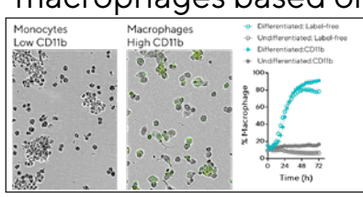
Histograms (top row) show the frequency of fluorescence intensity (left), Advanced Label-free Classification score (middle), or circularity (right) of Live and Dead control cells. Dotted lines indicate the threshold chosen for classification. Timecourses (bottom row) show time- and concentration-dependent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

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



Cells were seeded at 2K cells/well and incubated for 18h to assume their expected morphology. Cells were treated with campthothecin (0.1 – 10 µM), staurosporine (10 – 1000 nM) or cisplatin (0.5 – 50 µM) 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.

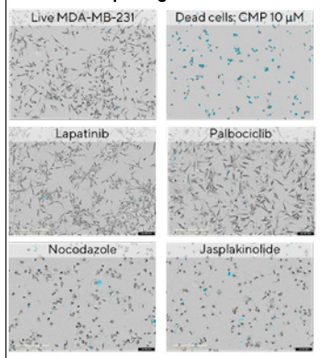
Label-free Differentiation Assay classifies macrophages based on morphology



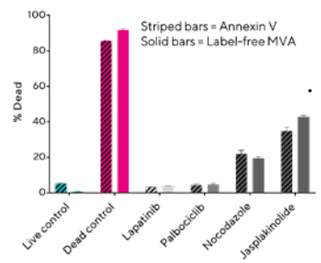
- THP-1 monocytes were differentiated ± PMA (100 nM) in the presence of FabFluor-488 labelled primary antibody for identification of the macrophage marker CD11b (green fluorescence).
- PMA induced differentiation of the monocytes to macrophages and cell morphology changed over time to an adherent cell 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).

Image-based morphology screening

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



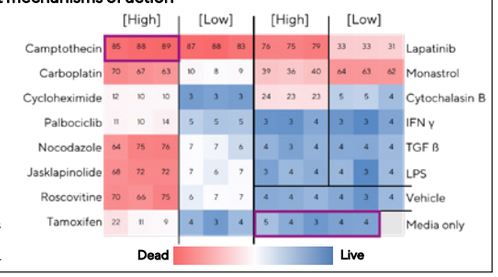
- MDA-MB-231 cells were seeded into a 96-well microplate (2K/well) in the presence of Annexin V and after 18h treated with compounds known to induce morphological changes.
- Live and dead control wells were included to train Incucyte® Advanced Label-free Classification.



- Images show cells at 72h post treatment displaying a range of altered morphologies.
- Bar graph shows % death at 110h post treatment. Advanced label-free classification of dead cells corresponds well to fluorescence classification across compounds.

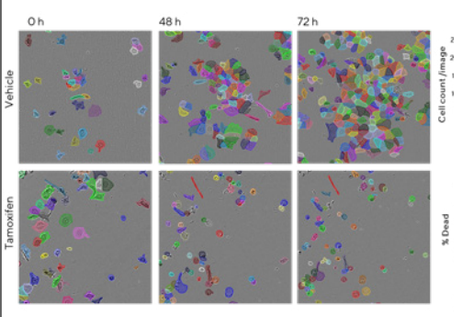
Label-free phenotypic screening based on cell morphology indicates % cell death induced by a range of compounds with different mechanisms of action

- AU565 cells were seeded into a 96-well plate at 2K cells/well. After 18h cells were treated with 2 concentrations of 14 compounds with varying mechanisms of action.
- Advanced label-free classification was performed using the control Dead (CMP, High) and Live (Media only, excluding H12 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.



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 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.



MCF7 receptor status:
ER+, PR+, Her2Low, CDK5/6-, EGFRLow, AR+

Compound targets:
Carboplatin: non-specific DNA intercalator
Lapatinib: Dual Her2/EGFR kinase inhibitor
Monastrol: Kinesin inhibitor
Palbociclib: CDK 4, 6 inhibitor
Roscovitine: CDK 5, 7, 9 inhibitor
Tamoxifen: ER inhibitor

Applications of Morphometry in Live-Cell Analysis

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Introduction

- Incucyte® Live-Cell Analysis Systems are ideal for long-term morphological analysis of live cells as they continuously acquire images from within an incubator without perturbing the cells
- AI-driven confluence measurements provide real-time information on cell growth and proliferation
- Cell morphology is highly complex and provides valuable information on cell viability and differentiation state. Incucyte® integrated Cell-by-Cell Analysis Software Module automatically segments individual cells after each image acquisition and provides morphological data for downstream analysis
- Multivariate data analysis (MVDA) enables simple and meaningful analysis of total cell morphology, eliminating reliance on a single variable
- We have developed a workflow for robust label-free classification of 2 user-identified populations based on segmented Phase Contrast HD images of cells (Incucyte® Advanced Label-free Classification Software Module)
- We demonstrate applications of this method for (i) quantification of % dead cells in a cytotoxicity assay (ii) of % macrophages in a differentiation assay, and (iii) detection of mitotic cells

Incucyte® Live-Cell analysis System

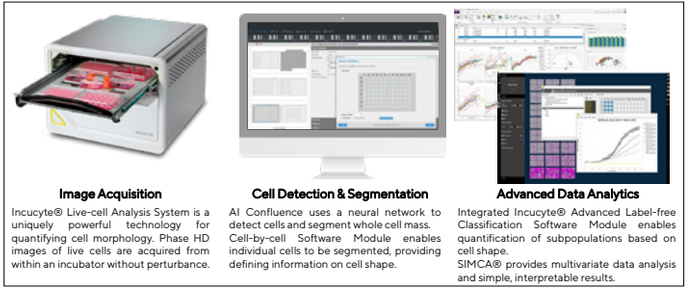


Image Acquisition

Incucyte® Live-cell Analysis System is a uniquely powerful technology for quantifying cell morphology. Phase HD images of live cells are acquired from within an incubator without perturbance.

Cell Detection & Segmentation

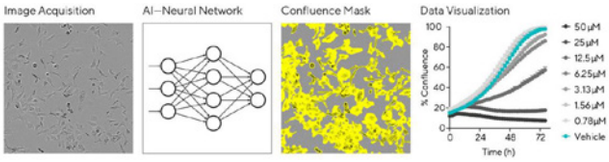
AI Confluence uses a neural network to detect cells and segment whole cell mass. Cell-by-cell Software Module enables individual cells to be segmented, providing defining information on cell shape.

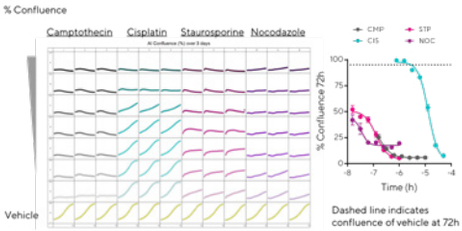
Advanced Data Analytics

Integrated Incucyte® Advanced Label-free Classification Software Module enables quantification of subpopulations based on cell shape.
SIMCA® provides multivariate data analysis and simple, interpretable results.

AI-driven cell detection for confluence analysis

Incucyte® AI Confluence is driven by a neural network pre-trained on a range of cell types with a wide variety of morphologies. The integrated software enables users to obtain real-time measurements of cell growth with minimal input. The neural network is highly adaptable and accurately segments numerous cell types with both healthy and dying or apoptotic morphology.



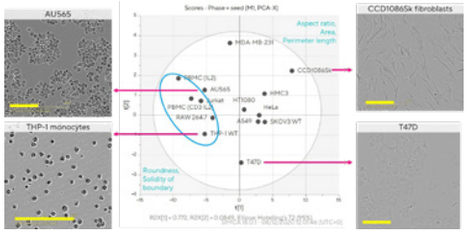


- A549 cells were treated with concentration ranges of 4 compounds.
- Phase contrast images were acquired every 2 hours for 3 days and automatically analysed using Incucyte® AI Confluence analysis.
- Microplate view shows that under vehicle conditions (bottom row) cells proliferate over time.
- In the presence of all 4 compounds, cell growth is inhibited.
- Concentration response curves show that compounds induce a concentration-dependent inhibition of cell growth.

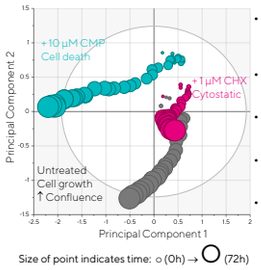
Cell segmentation enables quantification of morphology

Incucyte® morphological analysis with SIMCA®, a Sartorius multivariate data analysis package

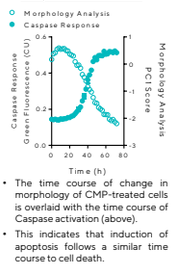
Integrated Incucyte® Cell-by-Cell Analysis Software module enables individual cells to be segmented. This provides data on cell count, fluorescence intensity within each cell, and also information on cell shape. Metrics including area, perimeter length, boundary solidity can be extracted for every cell. These metrics can be analyzed individually or using multivariate analysis techniques such as Principal Component Analysis (PCA). MVDA summarises multiple variables so that no valuable information is lost.



- Adherent and non-adherent cell types were segmented at 50% confluence, and 25 morphological features were extracted (ImageJ).
- Mean values per image were imported into SIMCA® for principal component analysis.
- PCA plot (left) shows that adherent cells cluster to the right while non-adherent cells (within blue circle) cluster to the left.
- Top right quadrant loading is driven by aspect ratio, area and perimeter length.
- Bottom left quadrant loading is due to roundness and boundary solidity.

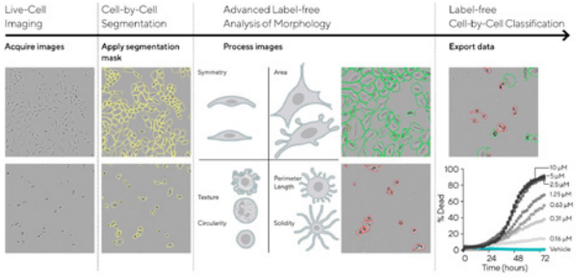


- A549 cells were seeded into a 96-well plate and treated with camptothecin (CMP, 10 µM, teal) cycloheximide (CHX, 1 µM, magenta) or vehicle control (untreated, grey).
- For each timepoint, mean values per image were imported into SIMCA® for principal component analysis.
- PCA plot shows (left) that healthy growing cells (grey) change shape over time as confluence increases.
- In the presence of cytostatic CHX (magenta), cells do not grow or increase confluence, and shape change is minimal.
- CMP treatment (teal) induces cell death causing cells to shrink and become highly textured. The PCA plot shows these diverging from healthy cell morphology over time (72h).



- The time course of change in morphology of CMP-treated cells is overlaid with the time course of Caspase activation (above).
- This indicates that induction of apoptosis follows a similar time course to cell death.

Integrated software enables simple and accurate classification based on morphology



Control wells are set up to generate images of 2 classes of different morphologies (e.g. Live vs Dead). Phase images are acquired without perturbance for the entire timecourse of an assay.

Cells are segmented in real time using integrated Incucyte® software.

The segmentation contains information on morphological features which can be extracted and analyzed using SIMCA®.

For integrated Advanced Label-free Classification, morphological data describing many variables (shape, texture, brightness) is distilled to a single axis using multivariate analysis.

A 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.

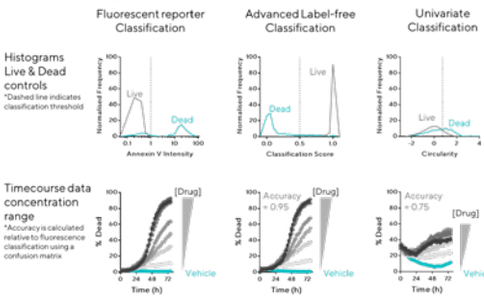
Setting a score threshold (e.g. 0.5) classifies cells as Class A or Class B (e.g. Live or Dead).

Classification masks identify the class of each cell in the image.

For label-free Live/Dead assays, % Dead cells can be calculated from Phase HD images without the use of fluorescent reagents.

Label-free Live/Dead Assay

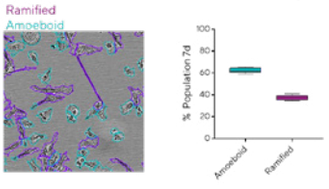
Incucyte® Advanced Label-free Classification of dead cells is comparable to the use of fluorescent cell health reagents and has higher accuracy than other label-free methods



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. Dashed lines indicate the threshold chosen for classification. Timecourses (bottom row) show time- and concentration-dependent increase in % Dead cells over time using each method.

- A549 treated with camptothecin (0.1 – 10 µM) in the presence of Annexin V.
- Phase 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.

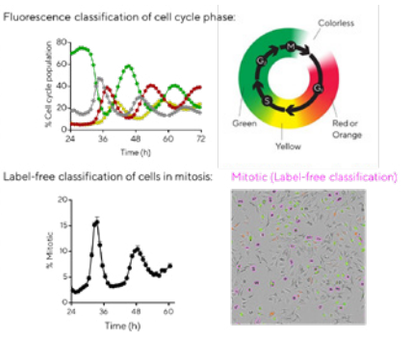
Label-free Differentiation Assay



- Primary monocytes were differentiated to macrophages by cytokine treatment over 7 days; the process was monitored within the Incucyte Live-cell Analysis System. Polarization of macrophages to M2 phenotype resulted in cells displaying a mixture of ramified and amoeboid phenotypes.
- Using the Advanced Label-free Classification workflow, individual cells within a heterogeneous image were selected to train a classification model.
- Segmentation masks (image – left) indicate classification results: ramified cells are outlined with a purple mask, amoeboid cells are outlined with a teal mask.
- Classification results (box chart – right) at 7 days post differentiation shows that approximately 60% of macrophages possessed amoeboid morphology indicating they are functionally active and phagocytic, while around 40% were in the resting state as indicated by their ramified morphology.

Mitotic cell detection

- To demonstrate complete analysis of the cell cycle using both fluorescence and label-free classification, HeLa cells expressing Incucyte® Cell Cycle were synchronised by treatment with Thymidine for 24h. At 24h the Thymidine was removed and the cells proceeded synchronously through the cell cycle.
- Images were acquired within the Incucyte® Live-Cell Analysis System, and individual cells were segmented using Incucyte® Cell-by-Cell Analysis Software.
- Cell cycle phase was determined by fluorescence classification of red, green, yellow and non-fluorescent cells.
- Time course of fluorescent cell population (top) demonstrates that at 24h a majority of cells are arrested in S/G2/M, and over time as the cells progress through the cycle peaks in other populations are observed.
- Mitotic cells were identified by their morphology using Advanced Label-free Classification.
- Time course of Advanced Label-free Classification (bottom) demonstrates the peaks in mitotic index through time. Image (bottom right) shows cells classified as mitotic outlined in a pink mask.

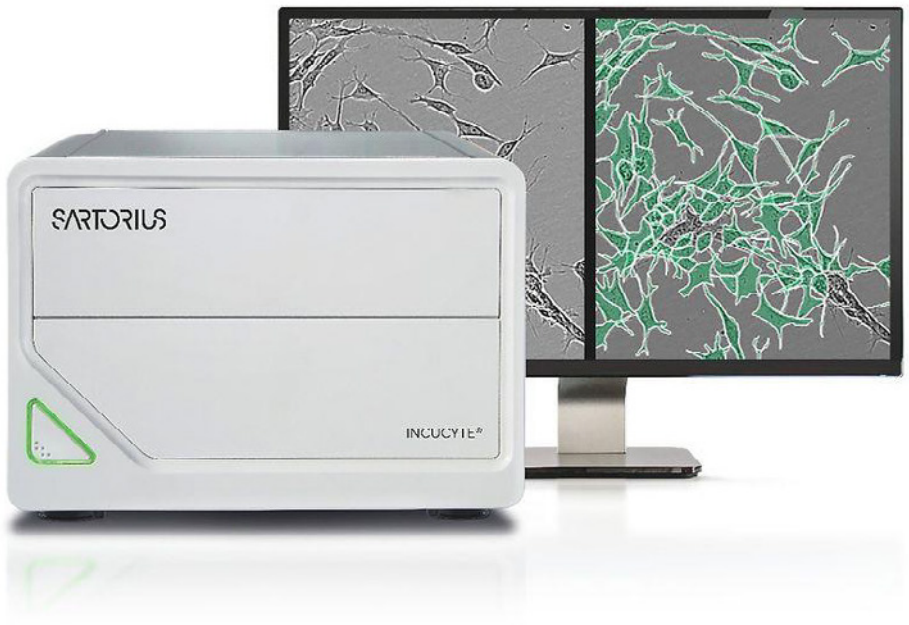


Summary & Conclusions

- Incucyte® Live-Cell Analysis Systems enable morphological analysis of live, unperturbed cells over extended periods of time.
- Integrated AI Confluence analysis provides real-time quantification of cell growth, while Cell-by-Cell segmentation identifies individual cells and provides valuable data describing cell morphology.
- SIMCA® provides user-friendly MVDA techniques for quantitative analysis of complex cell morphology. Principal component analysis (PCA) can be used to cluster similar morphologies, and quantify changes in morphology over time. Morphological cell death was shown to have a similar time course to Caspase activation, a marker of apoptosis.
- Incucyte® Advanced Label-free Classification analysis is a powerful, integrated tool for identification of cell subpopulations. Cells are classified into two user-defined groups with a simple workflow, enabling label-free quantification of cells based on morphology.
- We have successfully applied Advanced Label-free Classification analysis to several biological applications including Live/Dead assays across multiple cell types, differentiation of primary monocytes to a heterogeneous population of macrophages, and mitotic index calculations.

New Image-Based Data Set Transforms AI Label-Free Cell Segmentation

Artificial intelligence (AI) has been a game changer for automated processing of image content on a massive scale. Cell biologists are increasingly using it to analyze millions of cell images in a range of disciplines, including cancer biology. Advanced microscopy technologies are proving to be indispensable tools for expanding the applications of automated cell segmentation to a wider variety of cell culture models.



A Picture Is Worth A Thousand Insights

There is never a dull moment inside a living cell. Whether it is secreted growth factors, stress, or an oral therapeutic, our cells are always reacting to changes in their environment. Imaging cells and their subcellular organelles is crucial for helping us understand basic human biology, how diseases take hold, and how to combat them with effective treatments.

The scope of image-based cell research has always been limited by our capacity to analyze microscopy images at scale. Prior to advanced computational tools, this work was quite resource-intensive and heavily reliant on the subjective interpretation of well-trained experts. Unsurprisingly, progress was slow.

Man Vs. Machine

Recent breakthroughs in high-throughput microscopy and AI-enabled image analysis have empowered scientists to study biological phenomena on a whole other level. The insight such analysis provides is immense and it carries robust statistical power. In pharmaceutical research, such capabilities are a driving force for discovery by enabling fast *in vitro* drug screening and efficacy testing.

With automated cell segmentation, or cell identification, scientists can quantify discrete cellular features (e.g., cell type, division, shape) and characterize disease-associated phenotypes. Importantly, AI can reliably capture subtle changes over time or under varying conditions, that could otherwise be missed.

It Takes Training

Cell segmentation algorithms are “trained” using large, well-annotated imaging datasets. Most of the available algorithms, however, are designed for fluorescence-based cell imaging.

Growing evidence suggests that labels can interfere with the normal biology of cells. That’s why many researchers are ditching fluorescent probes for live-cell and label-free cell imaging approaches, which are more physiologically relevant and conducive to cell health. But there is one problem; well-annotated imaging datasets for this application remain scarce, or lack the morphological diversity seen in culture.

Go ahead, Be Label-free

The authors of a new study published in the journal Nature Methods created LIVECell (label-free in vitro image examples of cells) to help address this gap. LIVECell is a large, high-quality, expertly annotated dataset of phase-contrast cell images that can be used for training cell segmentation algorithms for biologically relevant cell imaging experiments.

They take advantage of the Incucyte® Live-Cell Analysis System for collecting 5,239 images, consisting of 1.6 million cells from diverse morphologies and culture densities. The Incucyte® Live-Cell Analysis System is designed specifically for performing continuous, non-perturbing live-cell analysis directly from the incubator, making it the ideal platform for this study.

The authors then used their dataset to train convolutional neural network-based models for single-cell applications and create a set of benchmarks to aid segmentation accuracy.

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Simplifying Progress

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

Gillian F. Lovell[1], Christoffer Edlund[2], Rickard Sjöegren[2], Daniel A. Porto[3], Nevine Holtz[3], Nicola Bevan[1], Jasmine Trigg[1], Johan Trygg[2], Timothy Dale[1], Timothy R. Jackson[1]*

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Introduction

- Light microscopy is a cost-effective, non-invasive, accessible modality for high-throughput live-cell imaging.
- Accurate segmentation of individual cells enables exploration of complex biological questions, 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 open-source, high-quality, 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.

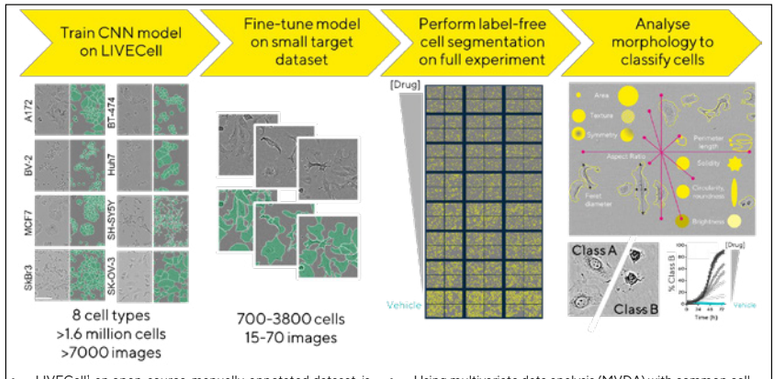
Incucyte® Live-cell imaging and analysis systems

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

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



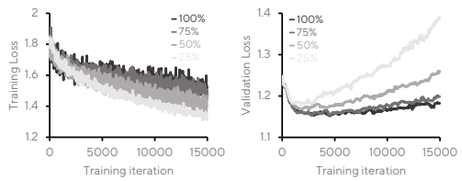
- LIVECell[1], an open-source, manually-annotated dataset, is used to generate a robust instance segmentation model trained to detect general cell features based on the CenterMask architecture[2].
- A small supplementary dataset is used to fine-tune LIVECell-trained models to learn cell features unique to a target application (e.g., differentiation, cell death).
- The final model is deployed to segment cells across a complete experiment.
- Using multivariate data analysis (MVDA) with common cell morphology metrics (e.g., size, intensity, texture and shape), we can measure morphological change in response to a treatment condition.

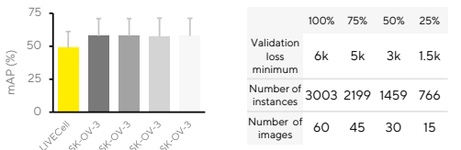
1. Edlund C*, Jackson TR*, Khalid N*, Bevan N, Dale T, Ahmed S, Trygg J, Sjögren R (in review). *Nature methods*. LIVECell: A large-scale dataset for label-free cell segmentation. *contributed equally. https://sartorius-research.github.io/LIVECell/
2. Lee Y & Park J (2020). *Proc. IEEECVF Conf. Comput. Vis. Pattern Recognit. CVPR*. CenterMask: Real-Time Anchor-Free Instance 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



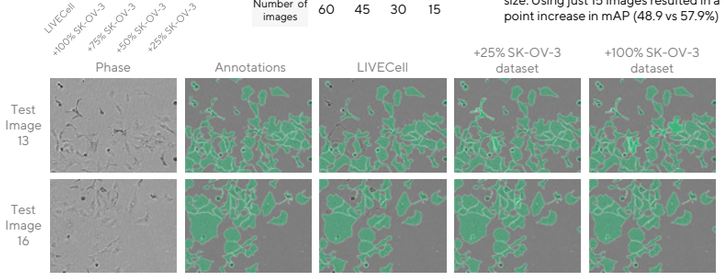


	100%	75%	50%	25%
Validation loss minimum	6k	5k	3k	1.5k
Number of instances	3003	2199	1459	766
Number of images	60	45	30	15

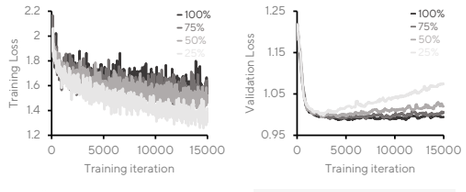
- 84 HD phase images of SK-OV-3 cells treated with the cytotoxic agent vinblastine were manually annotated.
- To explore the data requirements to fine-tune a LIVECell-trained model, a further 15K training iterations were performed using training sets of various sizes, using between 25-100% of the 60 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:

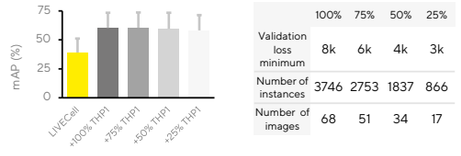
$$Total\ Loss = L_{class} + L_{center} + L_{bbox} + L_{mask}$$

- 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 9-point increase in mAP (48.9 vs 57.9%)



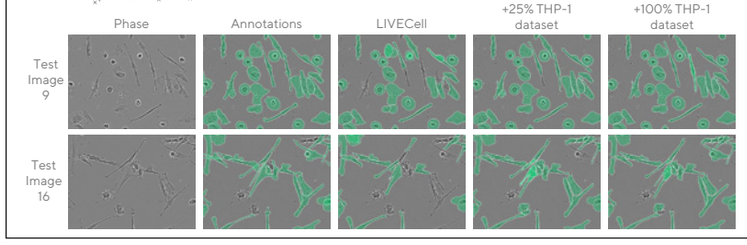
Example 2: THP-1 differentiation into macrophages





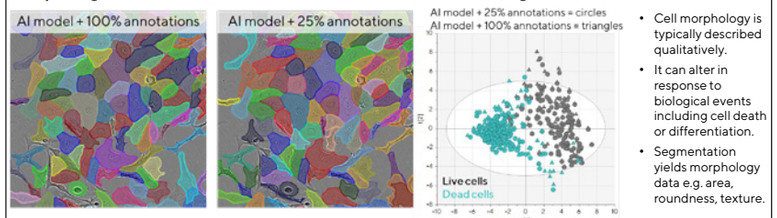
	100%	75%	50%	25%
Validation loss minimum	8k	6k	4k	3k
Number of instances	3746	2753	1837	866
Number of images	68	51	34	17

- 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.



Segmentation data provides biological insight

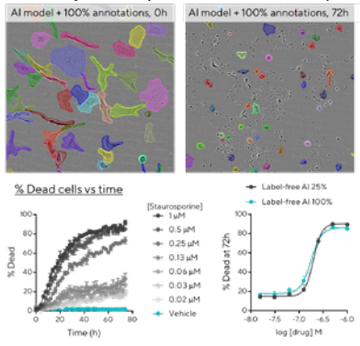
Morphological information on cells can be derived from the cell segmentations



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

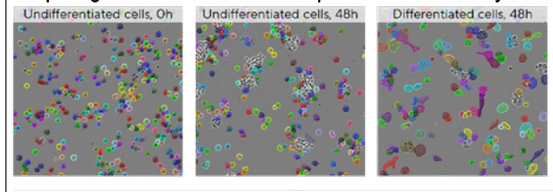
- Images show SK-OV-3 cells qualitatively similar segmentation performance using the model with 100% (left, +100%) or 25% additional annotated images (right, +25%). 25 metrics describing aspects of the morphology of every cell was extracted.
- Principal component analysis (PCA) of morphology metrics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

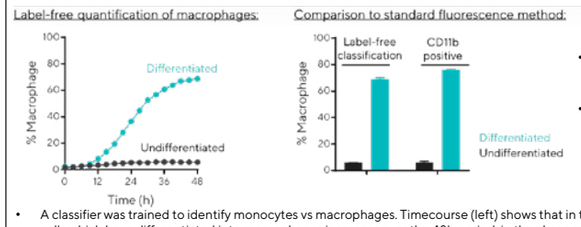


- SK-OV-3 cells were treated with the staurosporine (20 nM – 1 µM) to induce cell death. Cells were placed in an Incucyte® and Phase HD images were acquired every 2h for 3 days (images shown at 0h and 72h treatment).
- Cells were segmented and the morphological data extracted. Using a custom version of the Incucyte® 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 (pIC_{50} = 6.7).
- This demonstrates that robust AI-based segmentation enables highly accurate and label-free quantification of dead cells.

Morphological data enables label-free quantification of monocyte differentiation



- 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, 0h) 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 undifferentiated.
- 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 the requirement for fluorescence reagents and imaging.

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Simplifying Progress

AI-driven image analysis enables simplified, label-free cytotoxicity screening

Gillian Lovell[1*], Jasmine Trigg[1], Daniel Porto[2], Nevine Holtz[2], Nicola Bevan[1], Timothy Dale[1], Daniel Appledorn[2]

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[*] Corresponding author: askascientist@sartorius.com

Introduction

The increasing use of precious, patient-derived cells has driven the need for non-perturbing and label-free cell measurements. Incucyte® Live-Cell Analysis Systems enable long-term imaging of biology within a cell culture incubator to minimize cell disturbance. To gain insight into cell growth and viability label free, we developed the Incucyte® AI Cell Health Analysis Software Module which uses two deep neural networks to robustly segment cells and infer cell viability in a single step. These AI models were trained on a wide diversity of cell morphologies to ensure the analysis is applicable across a broad variety of tumor cell types.

1. AI Cell Health Analysis Workflow

AI Cell Health Analysis uses two trained convolutional neural networks (CNNs) for automatic segmentation and Live/Dead classification of cells. This analysis is label-free, requiring only Phase contrast images acquired using the Incucyte® Live-Cell Analysis System. The analysis provides user-friendly AI image analysis, enabling fast, accurate quantification of cell proliferation and viability.

Figure 1: Schematic diagram of the Incucyte® AI Cell Health Analysis Workflow. Phase contrast images are acquired and automatically processed. Cells are segmented and classified as Live or Dead; data can be visualized in real time for quantification of cell count and viability.

2. Validation of label-free AI Cell Health Analysis

The AI Cell Health CNNs were trained on a curated, diverse set of adherent and non-adherent cell lines, healthy and apoptotic. The resulting analysis accurately segments cells with wide-ranging morphologies (yellow outline, A), while the classification model yields label-free cell cytotoxicity data which is comparable with results obtained using standard fluorescent Annexin V reagent (A549 example shown, B). These validations were performed across 15 adherent and 7 non-adherent cell types.

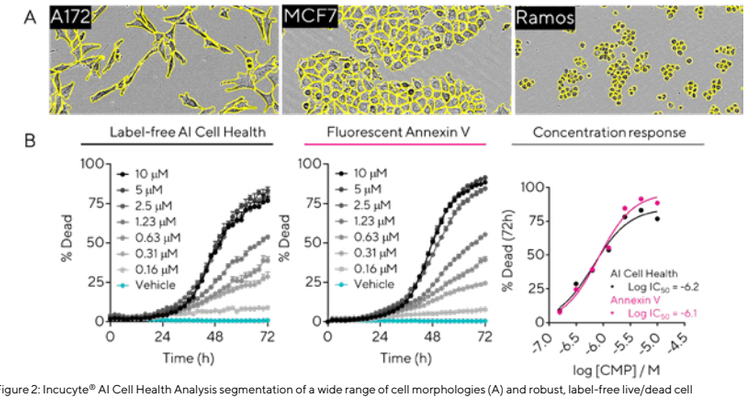
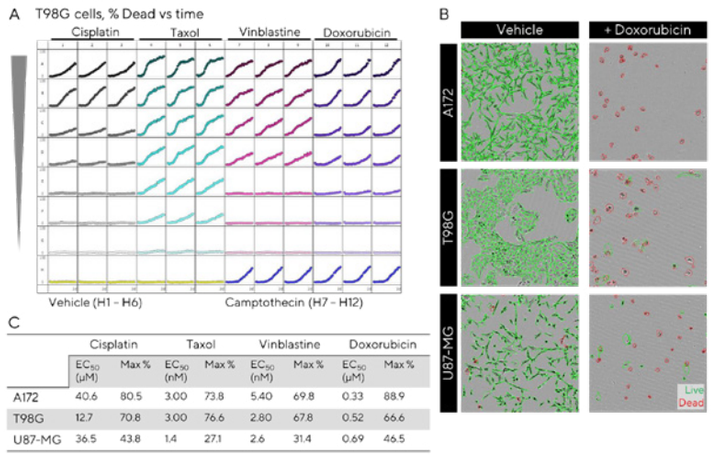


Figure 2: Incucyte® AI Cell Health Analysis segmentation of a wide range of cell morphologies (A) and robust, label-free live/dead cell classification (B). segmentation was validated using label-free cell count compared to fluorescent nucleus count of transfected cell lines (data not shown); classification was validated by comparison of label-free cytotoxic response to standard fluorescent readouts. Example shows A549 cells treated with a concentration range of camptothecin in the presence of Annexin V apoptosis reagent. Time courses of cell death calculated by label-free AI Cell Health (left) are similar to using a fluorescent Annexin V readout (middle) and calculated efficacy (right) are consistent.

3. Cytotoxicity in glioblastoma

Glioblastoma cells form highly aggressive tumors which are resistant to a range of chemotherapeutic compounds. Development of more robust treatments relies on accurate analysis of cell response to a range of drugs with varied mechanisms of action. Here we demonstrate how AI Cell Health Analysis can accurately measure cytotoxic effects of DNA-targeting cisplatin, microtubule-targeting Taxol and Vinblastine, and topoisomerase II inhibitor Doxorubicin on A172, T98G and U87-MG cells. The analysis accurately segments all 3 cell lines despite different morphologies and identifies live versus dead cells. This enables the effects of all 4 drugs to be compared across cell lines, indicating differential compound effects; plate views showing concentration ranges (A) provide an overview of drug efficacy.



C

	Cisplatin		Taxol		Vinblastine		Doxorubicin	
	EC_{50} (µM)	Max %	EC_{50} (nM)	Max %	EC_{50} (nM)	Max %	EC_{50} (µM)	Max %
A172	40.6	80.5	3.00	73.8	5.40	69.8	0.33	88.9
T98G	12.7	70.8	3.00	76.6	2.80	67.8	0.52	66.6
U87-MG	36.5	43.8	1.4	27.1	2.6	31.4	0.69	46.5

Figure 3: Cytotoxicity analysis in glioblastoma cell lines. Cells were treated with a concentration range of cisplatin, Taxol, Vinblastine and Doxorubicin. Plate view (A) shows the time course of % dead cells over time for T98G cells. Images (B) show cells 72h post treatment where green segmentation indicates live classification and red indicates dead classification. Table (C) displays EC_{50} values and maximal cell death.

4. CD20 mAbs induce direct apoptosis in Ramos B-cells

Anti-CD20 mAbs are used to facilitate antibody-dependent phagocytosis or cytotoxicity targeting B-cell lymphomas. These mAbs are also capable of inducing direct apoptosis in target cells. To assess the direct cytotoxic effect of anti-CD20 mAbs Rituximab and Truxima®, B-cell line Ramos were treated with increasing concentrations of mAb or IgG1 negative control. Image analysis using AI Cell Health determined that Rituximab and Truxima induce rapid concentration-dependent cell death, while non-binding IgG1 did not significantly increase the % dead cells per image.

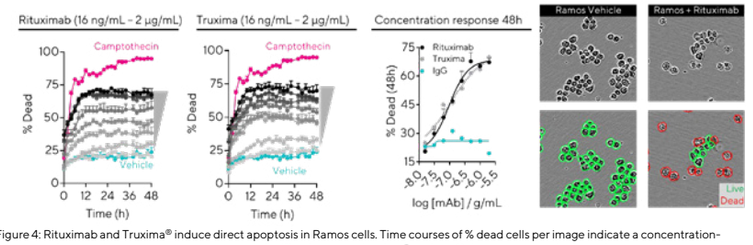


Figure 4: Rituximab and Truxima® induce direct apoptosis in Ramos cells. Time courses of % dead cells per image indicate a concentration-dependent increase in cell death upon treatment with Rituximab and Truxima®, while IgG (isotype control) induced little cell death. Concentration response curves indicate anti-CD20 antibodies possess similar efficacy. Images show Ramos B cells either untreated (left) or in the presence of Rituximab (right). AI Cell Health analysis performed on the Phase contrast images classed the cells as either live (green outline) or dead (red outline).

5. Differential cell death in breast cancer cell lines

Breast cancer cell lines present multiple, heterogeneous receptor expression patterns which affects their response to chemotherapeutics. Cells which over express estrogen receptor (ER) or epidermal growth factors (EGFR, HER2) enable development of receptor targeted drugs, increasing efficacy and specificity. Here we demonstrate the differential response of four breast cancer cell lines with diverse receptor patterning to Lapatinib (a dual HER2 and EGFR inhibitor) and Tamoxifen (ER inhibitor). AI Cell Health Analysis showed that Lapatinib induced cell death in HER2 positive AU565, BT474, and MCF7 but not triple negative MDA-MB-231; Tamoxifen was cytotoxic only to ER positive BT474 and MCF7.

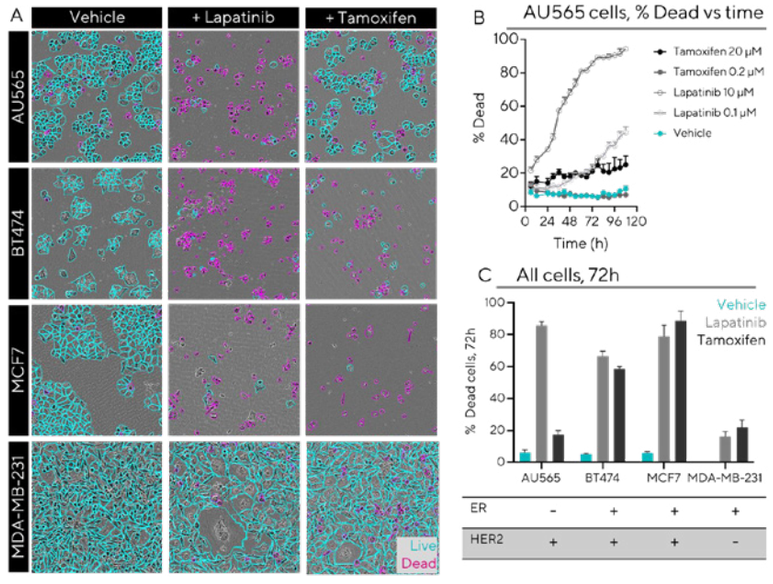


Figure 5: Chemotherapeutics Lapatinib and Tamoxifen target breast cancers with specific expression patterns. Cells were treated with Lapatinib (0.1, 10 µM) or Tamoxifen (0.2, 20 µM) and Phase contrast images were acquired every 2h for 5 days. Cytotoxicity was quantified using Incucyte® AI Cell Health Analysis. Images (A) show cells after 72h treatment with live (teal outline) or dead (magenta outline) classification shown. Time course of % Dead AU565 cells (B) shows that Lapatinib (open circles) induces rapid cell death at 10 µM and has a slower cytotoxic effect at 0.1 µM. High concentration of Tamoxifen (20 µM) has a minimal cytotoxic effect reaching a maximum of 20% cell death. Bar chart (C) compares % dead cells across all cell lines, showing that Lapatinib (10 µM) is cytotoxic to AU565, BT474, MCF7 but not MDA-MB-231 while Tamoxifen (20 µM) induces death only in BT474 and MCF7.

6. Summary & Conclusion

AI-driven image analysis has enabled scientists to derive more data from microscopy images. This information needs to be biologically relevant, interpretable, and easily processed by the user. Incucyte® AI Cell Health Analysis uses two CNNs to apply complex analytics to Phase contrast images and provides the user with a simplified workflow for quantification of pertinent information: cell count and viability (number and % of live and dead cells per image). Combined with long-term live-cell imaging methods, the Incucyte® enables accurate, adaptable cell segmentation and robust live/dead classification.
Here we have demonstrated the application of this method to three oncology models including cytotoxicity measurements of glioblastoma cell lines treated with compounds of different mechanisms of action, direct apoptosis of B cells induced by mAbs, and induction of cell death by chemotherapeutics targeting specific cell surface receptor patterns.

AI-Driven Label-Free Image Analysis for Screening Chemotherapeutic Cytotoxicity in Glial Cells

J. Trigg[1*], G. Lovell[1] , D. Porto[2], N. Holtz[2], N. Bevan[1], and T. Dale[1]

[1]Sartorius, Royston, UK; [2] Sartorius, Ann Arbor MI, USA
*Corresponding author: Jasmine.Trigg@sartorius.com

Summary & Impact

- Glioblastoma multiform (GBM) is a malignant brain tumour associated with poor prognosis. To progress effective chemotherapies there is a need to develop more translational models which enable increased insight into cytotoxic effects in a non-perturbing manner.
- Here we demonstrate a robust *in vitro* assay using the Incucyte® Live-Cell Analysis System and integrated software to assess cytotoxic effects of clinically relevant chemotherapeutics in established GBM cell lines and primary astrocytes in 96-well plates.
- We utilized the Incucyte® AI Cell Health Analysis Software Module, which is driven on pre-trained neural networks (CNN), and is a robust solution for label-free cell segmentation and Live/Dead classification.
- We observed differential time- and concentration-dependent effects across glial cell types.
- These data exemplify that Incucyte® Live-Cell Analysis System, alongside advanced AI-driven analytics, is a powerful approach for assessing cytotoxicity in glial cell types and is amenable to screening of therapeutic candidates.

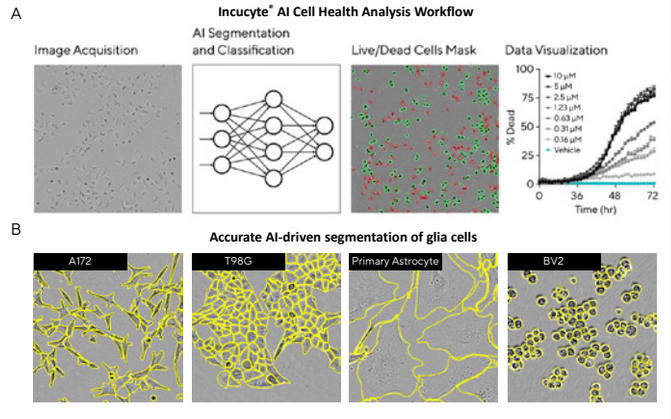
Incucyte® Live-Cell Analysis Solutions

Incucyte® Live-Cell Analysis System
A fully automated HD phase-contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

Incucyte® Software
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

Incucyte® Reagents and Consumables
A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted fluorescent proteins for cell counting plus no-wash cell health reagents for apoptosis, cytotoxicity, and many more.

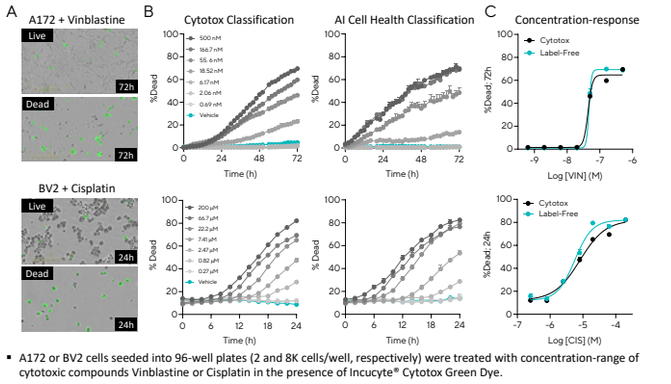
Assay Principle



A. Incucyte® AI Cell Health Analysis workflow. HD phase contrast images are acquired and processed using pre-trained neural networks to automatically segment and classify cells as live or dead. This AI-driven analysis is applied to all wells and timepoints, providing robust data and visualization of live or dead masks.

B. The AI Cell segmentation is highly accurate even in confluent images and adapts to a multitude of cell morphologies. Shown is the AI segmentation applied to glia cell types, including GBM cell lines A172 and T98G, primary rat cortical astrocytes, and semi-adherent BV2 microglia.

AI-driven Live/Dead classification validation



- A172 or BV2 cells seeded into 96-well plates (2 and 8K cells/well, respectively) were treated with concentration-range of cytotoxic compounds Vinblastine or Cisplatin in the presence of Incucyte® Cytotox Green Dye.

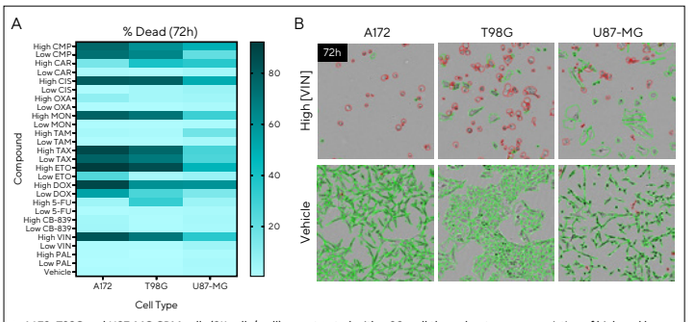
A. Phase HD and fluorescence images were acquired in an Incucyte® Live-Cell Analysis System and representative live and dead images are shown.

B. Cell death was quantified using Incucyte® AI Cell Health Live/Dead classification (label-free analysis) and fluorescence classification of Cytotox positive cells. Time courses show comparable time- and concentration-dependent increase in the % dead cells.

C. Corresponding concentration-response curves show comparable EC_{50} values are obtained using these classification models.

All data presented as Mean ± SEM, N = 3 – 6 replicates.

Chemotherapeutic cytotoxicity screen

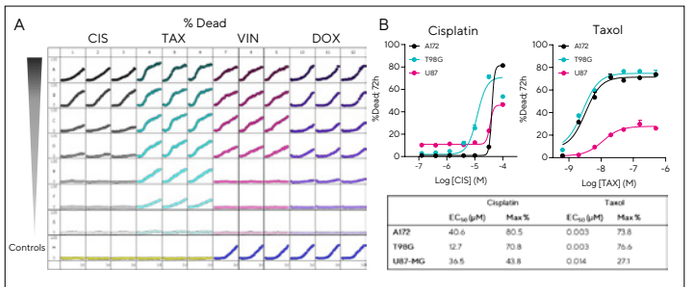


- A172, T98G and U87-MG GBM cells (2K cells/well) were treated with a 96-well throughput screen consisting of high and low concentrations of 13 chemotherapeutics. Images were acquired in the Incucyte® over 3 days and quantified using Incucyte® AI Cell Health Analysis Software Module.

A. Heatmap shows % dead cells at 72h for all compounds and cell types. Observed that U87-MG shows reduced sensitivity for most compounds.

B. Representative phase images show Live/Dead classification masks for high vinblastine treated cells and vehicle and highlight adaptability of segmentation and classification across different live and dead morphologies. Data indicates differences in potency for vinblastine across glioblastoma types.

Comparing concentration-dependent cytotoxicity in GBM cells



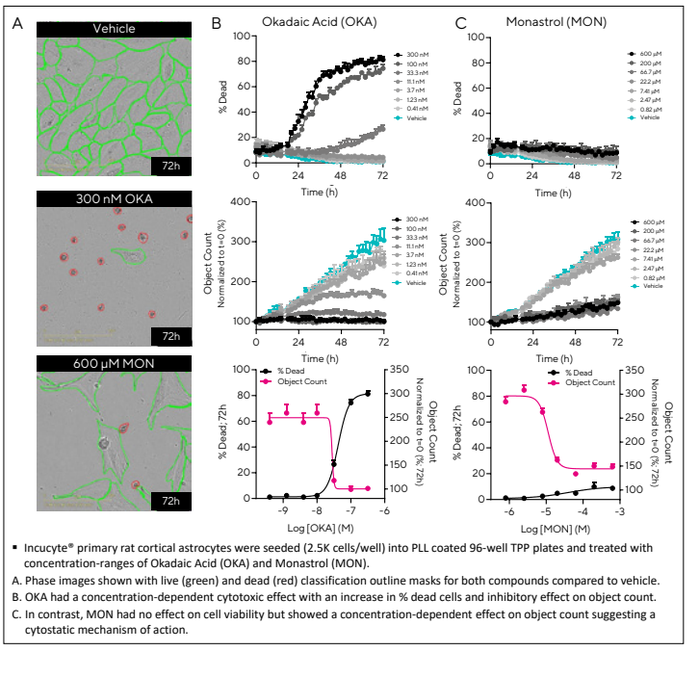
	Cisplatin		Taxol	
	EC_{50} (µM)	Max %	EC_{50} (µM)	Max %
A172	40.6	80.5	0.003	73.8
T98G	12.7	70.8	0.003	76.6
U87-MG	36.5	43.8	0.014	27.1

- A172, T98G, and U87-MG cells were seeded (2K cells/well) and treated with concentration-ranges of 4 chemotherapeutics.

A. Microplate graph displays % dead over 3 days for T98G cells, with varying kinetic concentration-dependent effects observed.

B. Concentration-response curves shown for Cisplatin (CIS) and Taxol (TAX) which reveal a cell-type dependent differences in compound sensitivities with U87-MG showing reduced potency compared A172 and T98G cells for both compounds. EC_{50} values and maximal % cell death are shown in table.

Cytotoxic vs. cytostatic effects in primary astrocytes



- Incucyte® primary rat cortical astrocytes were seeded (2.5K cells/well) into PLL coated 96-well TPP plates and treated with concentration-ranges of Okadaic Acid (OKA) and Monastrol (MON).

A. Phase images shown with live (green) and dead (red) classification outline masks for both compounds compared to vehicle.

B. OKA had a concentration-dependent cytotoxic effect with an increase in % dead cells and inhibitory effect on object count.

C. In contrast, MON had no effect on cell viability but showed a concentration-dependent effect on object count suggesting a cytostatic mechanism of action.

SARTORIUS

Simplifying Progress

Multi-Parametric Quantification of Monocytes using Live-Cell Analysis

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*Corresponding author: Jasmine.Trigg@sartorius.com

Introduction

- Monocytes play critical roles in innate immunity by migrating to inflamed tissue, where they clear micro-organisms and apoptotic cells, repair injured tissues, and recruit other immune cells.
- These highly plastic cells can change their functional phenotypes in response to a variety of cellular signals, including the Akt signaling pathway.
- Here we provide robust *in vitro* assays for the kinetic evaluation of monocytes and exemplify how these further our understanding of their biological roles.
- The Incucyte® Live-Cell Analysis System was used to acquire phase and fluorescence images of monocytes, which were automatically analyzed using integrated software.
- Cytokine analysis was performed using the iQue® Advanced Flow Cytometry platform and a custom Qbeads® PlexScreen Kit.
- These data exemplify that live-cell analysis, alongside advanced analytical methods, is a powerful approach enabling multi-parametric quantification of immune cells.

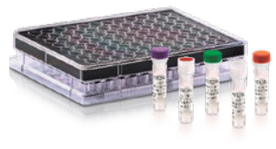
Incucyte® Live-Cell Imaging and Analysis Solutions

Incucyte® Live-Cell Analysis System

A fully automated HD phase-contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

Incucyte® Software

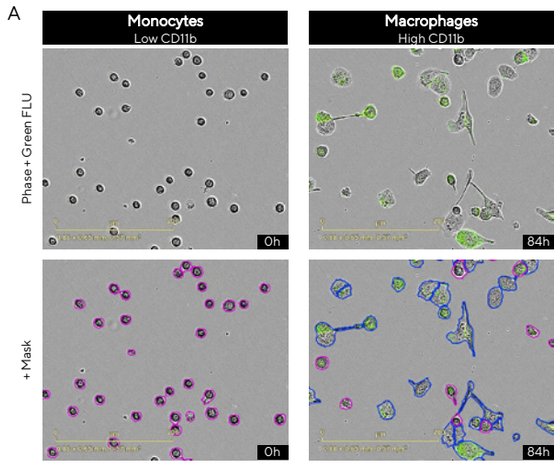
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

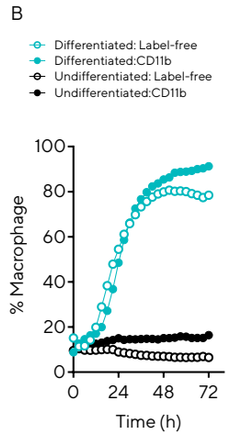


Incucyte® Reagents and Consumables

A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted fluorescent proteins for cell counting plus no-wash cell health reagents for apoptosis, cytotoxicity, and many more.

Label-free differentiation assay classifies macrophages based on morphology



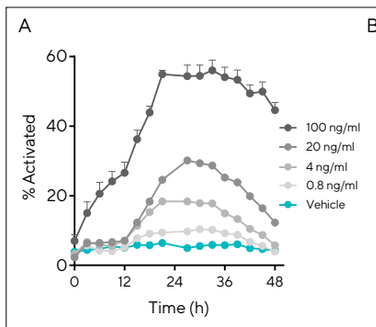


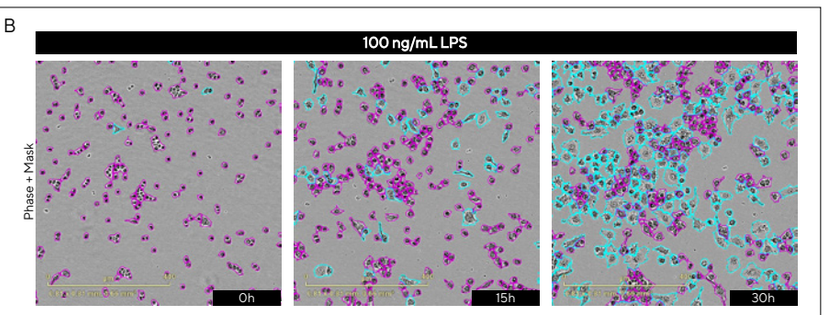
- THP-1 monocytes were differentiated with PMA (100 nM) in the presence of Incucyte® Fabfluor-488 labeled CD11b (macrophage marker). HD images were acquired using the Incucyte® Live-Cell Analysis System over 3 days.
- PMA induced changes in cell morphology over time to an adherent phenotype with a concurrent increase in CD11b expression.

A. Incucyte® Advanced Label-Free Classification Analysis was used to classify macrophages based on morphology. Phase HD and fluorescence images show CD11b expression and classification masks for monocytes (pink) and macrophages (blue) at 0 and 84 hours.

B. Time course shows the increase in % macrophages using the label-free method (open circles) is comparable to CD11b expression (fluorescence – closed circles).

Label-free morphological assessment of monocyte activation





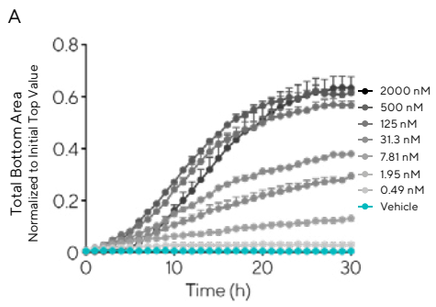
- RAW monocytes were stimulated with increasing concentrations of LPS and phenotypic morphological changes over 2 days were quantified using Incucyte® Advanced Label-Free Classification Analysis.

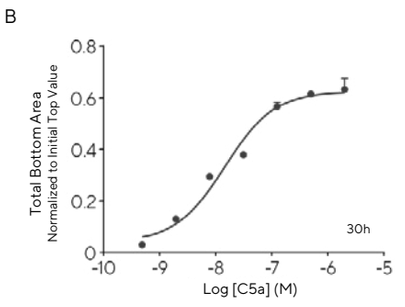
A. Time course shows LPS-induced a concentration-dependent increase in % activated as cells become adherent, larger and flatter, with a maximal response of 56% for 100 ng/mL at 33 hours.

B. Representative images of observed rapid changes in morphology following LPS stimulation. Shown are classification masks for monocytes (pink) and activated macrophage-like cells (cyan) over 30 hours.

Biological significance of monocyte maturation in chemotactic migration

- THP-1 cells, either naïve or PMA differentiated, were seeded on Incucyte® Clearview Plates coated with fibronectin and exposed to chemoattractant C5a.
- Time course (A) and transformed data at 30 hours (B) show differentiated macrophage-like THP-1 cells had a concentration-dependent chemotactic response towards C5a, whilst little-to-no response was observed in naïve THP-1 cells (data not shown).
- Results reveal functional significance of monocyte maturation in chemotactic migratory responses.





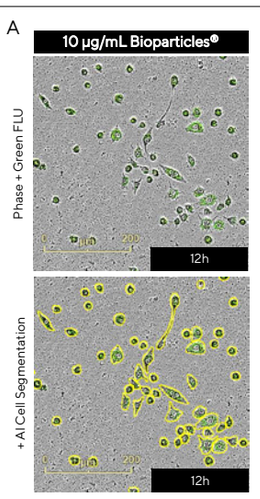
Artificial Intelligence (AI) driven analysis of phagocytic monocytes

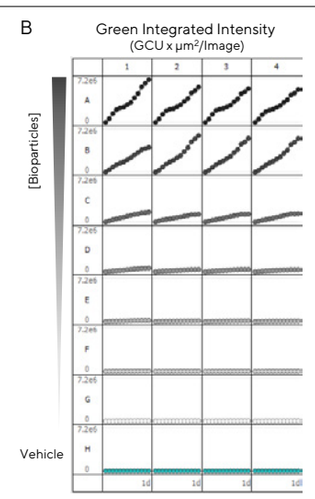
- RAW monocytes were treated with pHrodo® *E.coli* Bioparticles® for Incucyte® and analyzed using Incucyte® AI Cell Health Analysis.

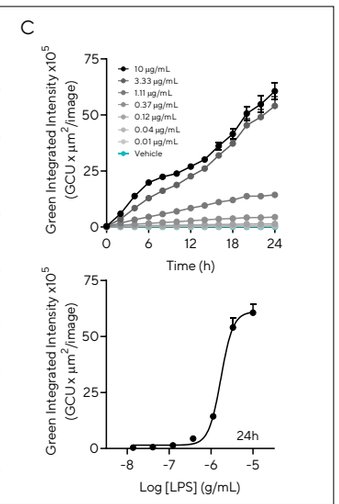
A. Representative images of monocytes; non-engulfed bioparticles are visible in the phase image whilst engulfed bioparticles fluoresce green. AI Cell Segmentation shows accurate masking of cells alone (yellow).

B. Microplate graph reports increasing green fluorescent intensity within segmented cell boundary over 24 hours and highlights assay robustness.

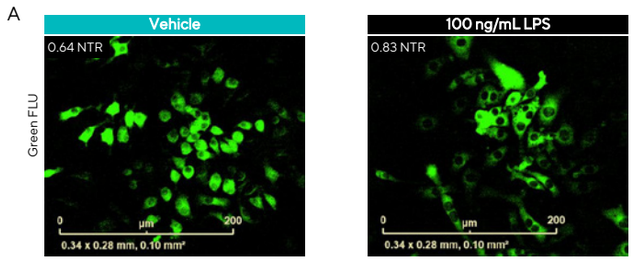
C. Observe a kinetic concentration-dependent increase in cellular fluorescence as bioparticles are engulfed by monocytes.

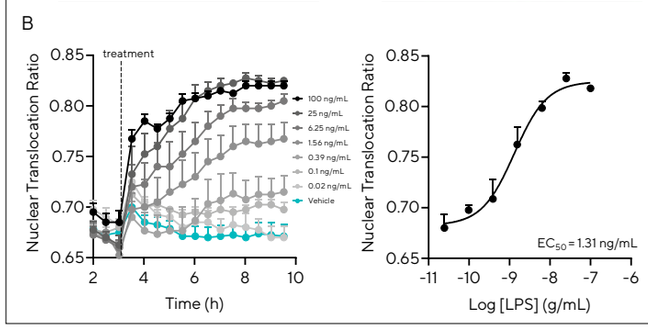


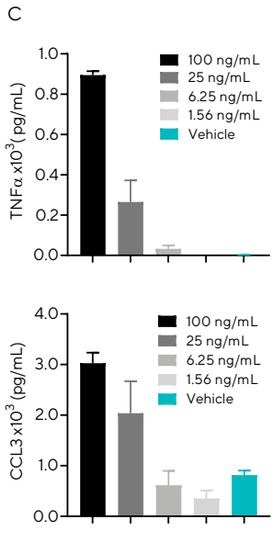




Activation of Akt in Lipopolysaccharide (LPS) stimulated monocytes







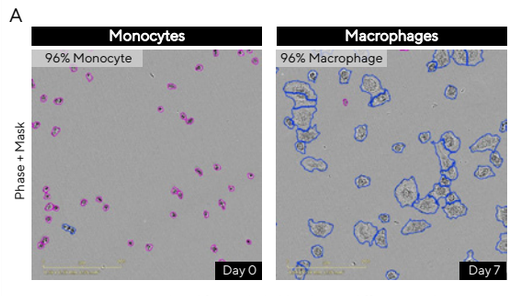
- RAW monocytes expressing Incucyte® Kinase Akt biosensor were serum-starved for 3h to inactivate Akt then treated with a concentration range of (LPS).
- Images and data shown at 6 hours post-treatment.

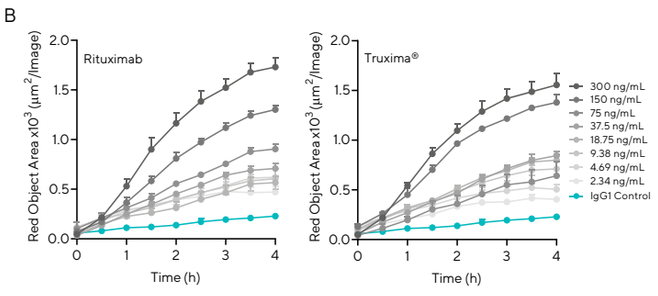
A. Green fluorescence images show cytoplasmic translocation for LPS compared to vehicle.

B. Graphs reveal LPS induced a rapid concentration-dependent increase in nuclear translocation ratio (NTR) indicating Akt activation.

C. Supernatant analysis was performed using the iQue® and a Qbeads® PlexScreen Kit. LPS stimulated release of inflammatory proteins TNFα and CCL3 (MIP-1α), consistent with LPS inducing activation via the release of pro-inflammatory agents.

Anti-CD20 mAbs promote antibody-dependent cellular phagocytosis in macrophages





- Primary monocytes isolated from whole blood were differentiated into macrophages (M0) using cytokines. pHrodo® for Incucyte® labeled Ramos cells were treated with monoclonal antibodies (mAbs) targeting anti-CD20, Rituximab or Truxima, or IgG isotype control (300 ng/mL) and co-cultured with macrophages.

A. Macrophage differentiation was monitored and quantified using Incucyte® Advanced Label-Free Classification Analysis, images show classification masks for monocytes (pink) and macrophages (blue) on day 0 and day 7.

B. Time courses show Rituximab and Truxima® induced ADCP in a concentration-dependent manner, with similar kinetic profiles being observed over 4 hours.

Data presented as mean ± SEM, n = 3 - 6 replicates.

Featured products

Incucyte® Live-Cell Analysis Systems
by Sartorius Group

"Great Results, Very much satisfactory"

Ease of use: ★★★★★ **After sales service:** ★★★★★ **Value for money:** ★★★★★

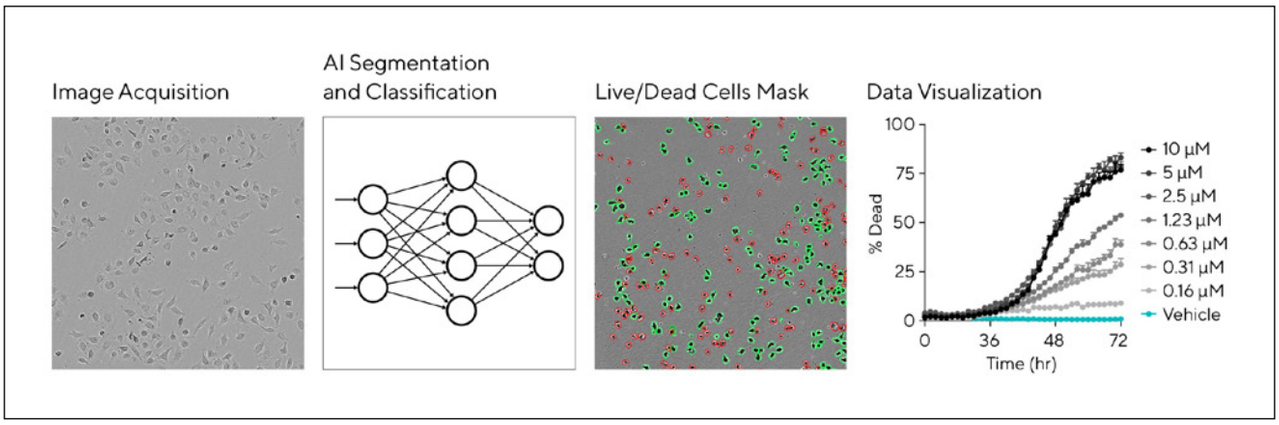
Rating: ★★★★★

Application Area: Analysis of Live Tissues

"The Incucyte Live-Cell Analysis System is truly a game-changer in the field of cell biology research. I have had the opportunity to work with this system and I must say, it has revolutionized the way we study live cells. The user interface of the Incucyte software is intuitive and user-friendly. Setting up experiments and analyzing data is a breeze, even for those who may not have extensive experience with imaging systems. The system provides robust and reliable data, enabling us to make informed decisions and draw meaningful conclusions from our experiments."

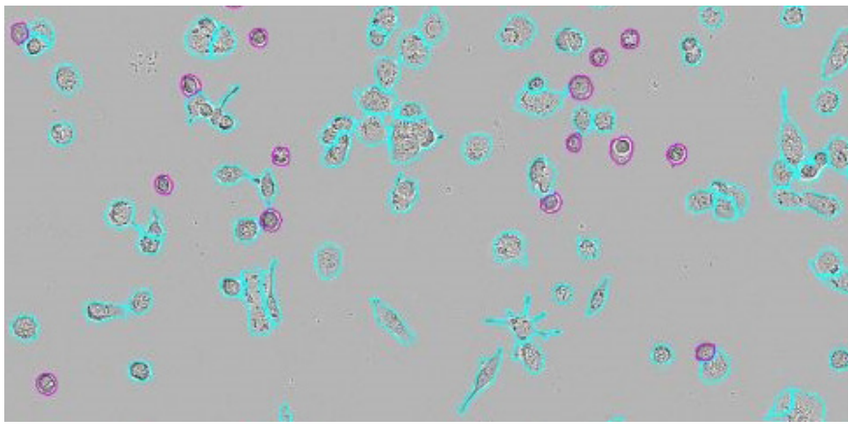
Antik Chakraborty, Vellore Institute of Technology

Incucyte® AI Cell Health Analysis Software Module
by Sartorius Group



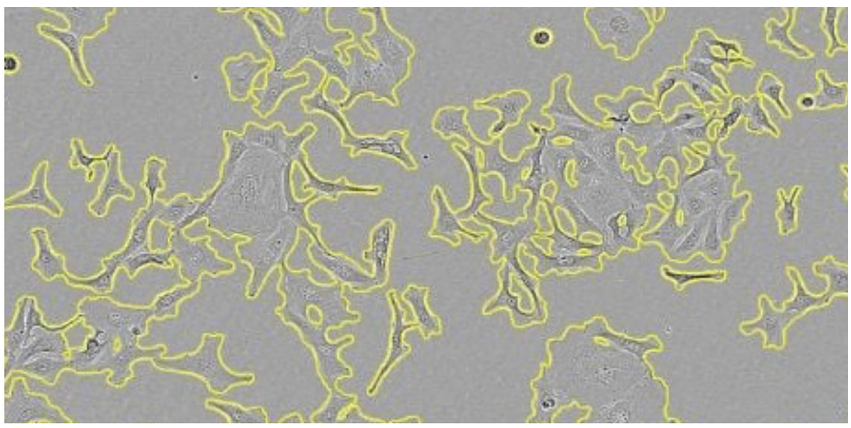
The Incucyte® AI Cell Health Analysis Software Module is an all-in-one analysis tool that segments cells and classifies them as live or dead. It allows users to perform cell health analyses without the need for fluorescent labels, generating accurate and objective data with reduced time requirements and cost.

Incucyte® Advanced Label-Free Classification Analysis Software Module
by Sartorius Group



Monitor adherent cell morphology changes objectively to determine live/dead cell counts or classify cells based on morphology via label-free multivariate analysis.

Incucyte® AI Confluence Analysis
by Sartorius Group



Non-invasive, image-based measurements of cell growth based on area (confluence) or cell number (count) metrics, visually verified via images and movies.

Additional resources

eBook

In this eBook, discover further applications that utilize the complete suite of Incucyte live-cell instruments, assays, reagents, and integrated software analysis tools. The guide features the new, AI-driven software on the Incucyte® Live-Cell Analysis Systems and a new chapter on kinetic biosensor assays for analyzing cancer cells, including a kinase Akt Assay.

Live-cell analysis handbook: A guide to real-time live-cell imaging and analysis »