Quantifying immune cell subsets in living cultures over time using IncuCyte® live-cell analysis

C. Szybut, N. Bevan, H. Campwala, L. Kelsey, N. Dana, T. Jackson, N. Holtz, E. Endsley, T. Dale, D. Trezise
Essen BioScience, Welwyn Garden City, AL7 3AX UK & Ann Arbor, MI, 48108, USA

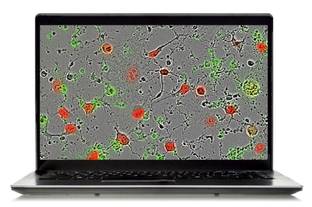
Summary & Impact

- Heterogeneity exists in all cellular populations, ranging from the cell types present to differences at the genetic level or stage of cell cycle. This heterogeneity plays an important role in how populations react in response to therapeutics and biological stimuli.
- To date IncuCyte® analysis has been solely based on population-averaged measures whereby object (cell) data is consolidated into an aggregate metric.
- However, effects on subpopulations can sometimes be masked by larger numbers of 'non-responsive' cells or similar sized populations may produce opposite responses that result in a net zero result.
- Analysis at the cell-by-cell level promises valuable and additional biological insight beyond which whole population measures may deliver.
- The IncuCyte cell-by-cell software module and FabFluor-488 labelling protocol provides automated image capture and analysis in real time in order to provide an integrated solution for monitoring at the cell-by-cell level and increase biological insight.
- Here, we present data validating the cell-by-cell analysis segmentation and classification and use examples of PBMC activation to show parameter changes over time. Importantly, these changes can be related to function.

IncuCyte® System for Continuous Live-cell Analysis: Methodology

IncuCyte® S3 Live-Cell Analysis System

A fully automated phase contrast and two-color fluorescence imager 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.

NEW: IncuCyte® S3 Cell-by-Cell Software Module

IncuCyte® Reagents and Consumables

A suite of non-perturbing cell labeling and reporter reagents. Includes nuclear-targeted GFP and RFPs for cell counting plus no-wash cell health reagents for apoptosis and cytotoxicity.

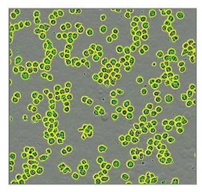
1 PREPARE ASSAY PLATES

Seed cells, add treatments and optional non-perturbing reagents.

2 ACQUIRE IMAGES

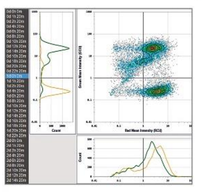
Acquire images with 20x objective, at appropriate time interval and length for assay.

3 IDENTIFY OBJECTS



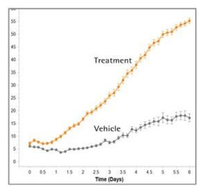
Segment image and create a mask that delineates cell boundaries.

4 MEASURE and CLASSIFY



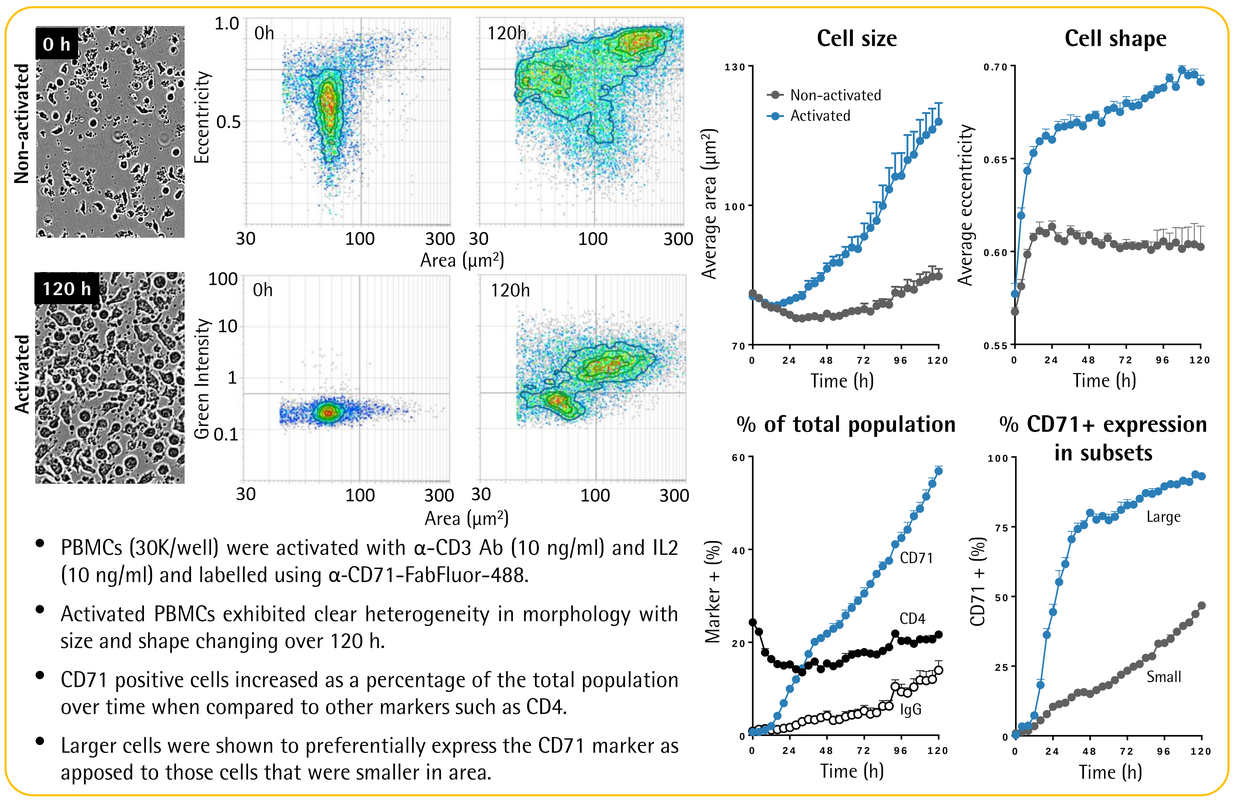
Generate metrics for each cell in the mask. Classify cells into subsets based on shape, size or fluorescence properties.

5 VISUALIZE DATA



Graph changes in subsets over time.

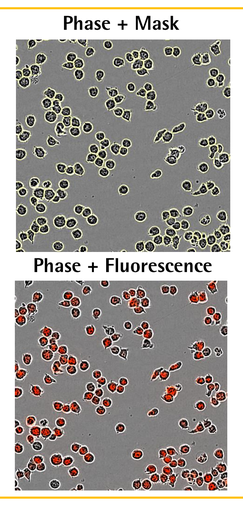
Monitoring PBMC activation: morphology & protein expression

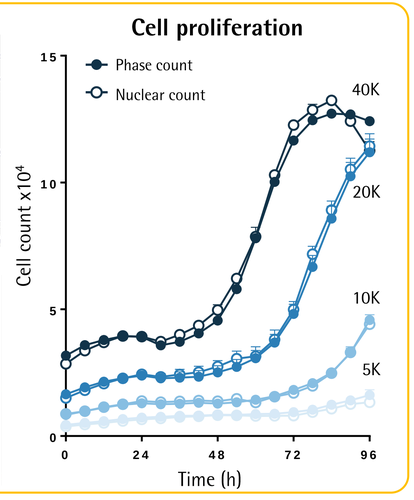


- PBMCs (30K/well) were activated with α-CD3 Ab (10 ng/ml) and IL2 (10 ng/ml) and labelled using α-CD71-FabFluor-488.
- Activated PBMCs exhibited clear heterogeneity in morphology with size and shape changing over 120 h.
- CD71 positive cells increased as a percentage of the total population over time when compared to other markers such as CD4.
- Larger cells were shown to preferentially express the CD71 marker as apposed to those cells that were smaller in area.

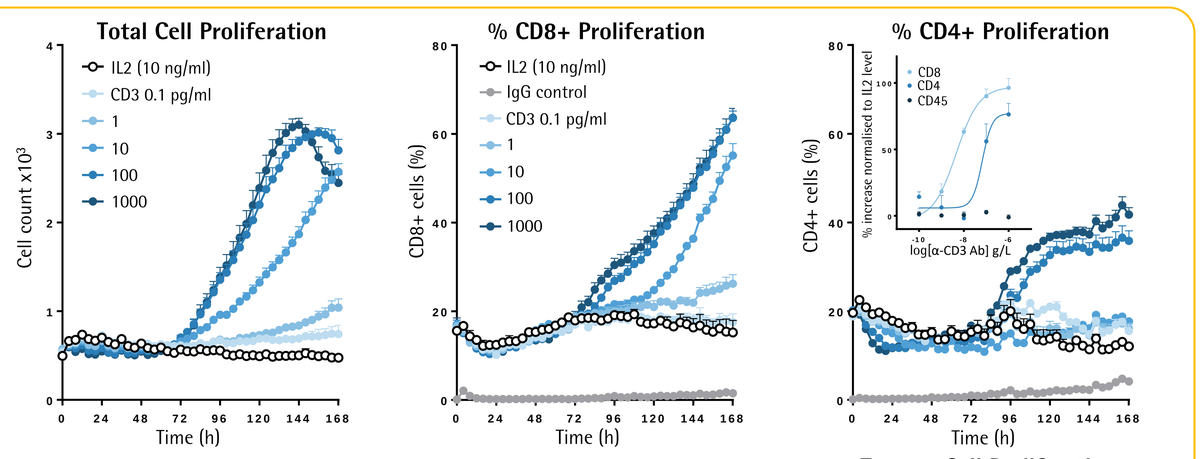
Label-free, accurate non-adherent cell segmentation

- NucLight red labelled Jurkat cells were plated at various densities (2.5-50K/well) and cell numbers were monitored over a 96 h period.
- Label-free and nuclear count analysis were performed.
- Masking using the cell-by-cell analysis shows accurate identification of cell boundaries.
- Time courses showing cell count for each analysis are highly comparable.
- Data sets overlay for all cell densities and all time points.

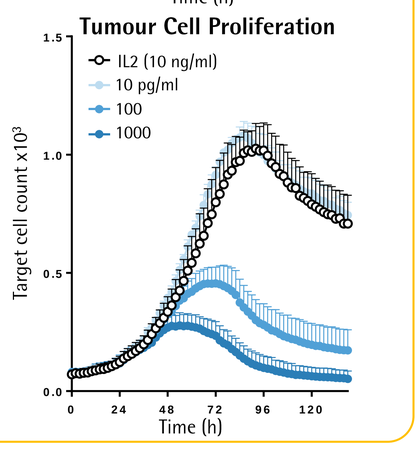




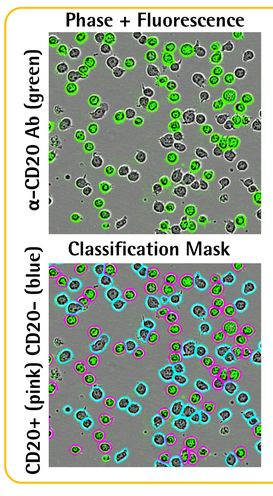
Linking PBMC subset proliferation to immune cell killing

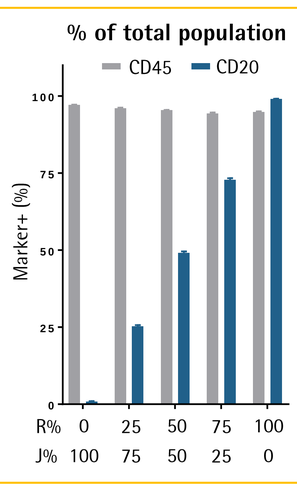


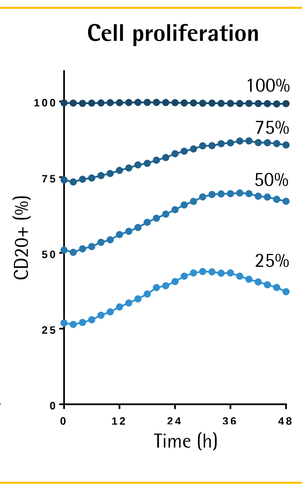
- PBMCs (30K/well) were labelled with either α-CD8 or α-CD4 Abs using FabFluor-488 reagent and incubated with IL2 (10 ng/ml) and increasing concentrations of CD3 Ab (0.1-10000 pg/ml).
- α-CD3 Ab induced total PBMC proliferation in a concentration-dependent manner.
- The CD8+ subset proliferation was significantly enhanced to >60% of the total population in response to α-CD3 Ab stimulation.
- The CD4+ subset was less responsive to α-CD3 Ab treatment where potency was reduced 10-fold when compared to the CD8+ response.
- Using the same stimulation conditions in an immune cell killing assay shows that target cell death temporally aligns with CD8+ cell enrichment.
- Enrichment of the CD8+ population serves to attack and kill target cells.



Specific, non-perturbing fluorescent labelling



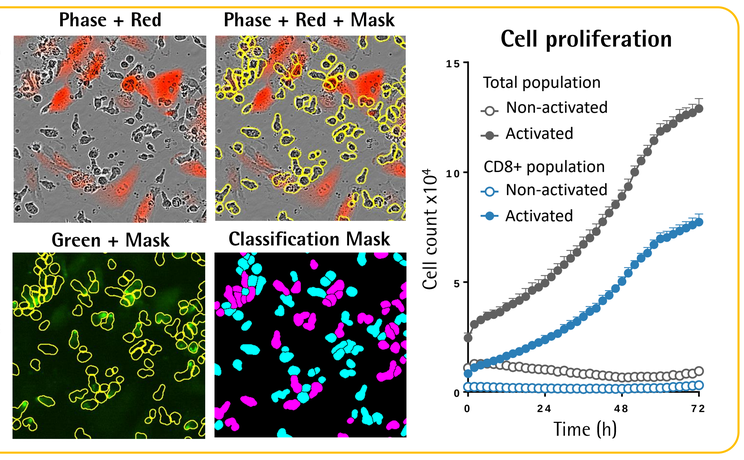




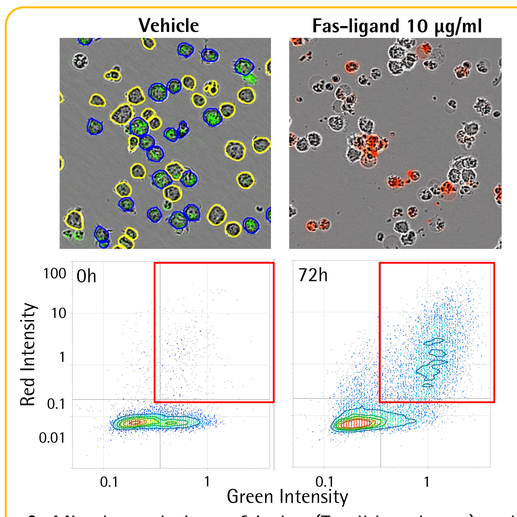
- Jurkat and Ramos cell mixtures (0-100%) were plated and monitored over a 48 h period.
- α-CD20 Ab was fluorescently labelled with IncuCyte FabFluor-488 reagent and used to identify the Ramos cells.
- Initial CD20+ subset percentages were comparable to the percentage of Ramos cells initially plated.
- CD45 is present on both cell types and was represented by 100% of the total population.
- The CD20+ subpopulation could be tracked over time.

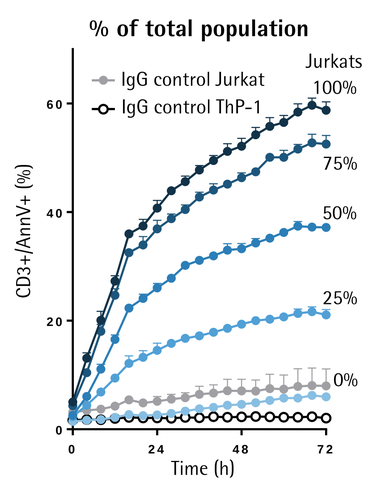
Monitoring heterogeneity in the tumour microenvironment

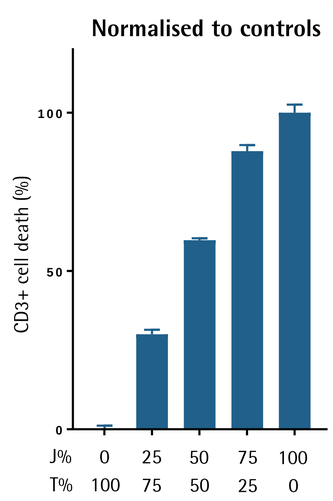
- A co-culture of A549 CytoLight red cancer cells and PBMCs were plated and incubated with α-CD8-FabFluor-488 conjugate and IL2/CD3.
- PBMCs were segmented and analysed, A549 cells were excluded from analysis.
- Total PBMC and CD8+ T cell subpopulation proliferation was able to be monitored.
- Numbers of CD8+ cells expressed as a percentage of the total PBMC population increased from 35% to 60% which exemplifies a significant enrichment of CD8+ cells.
- Classification mask gives information on CD8+ (pink) and CD8- (blue) proximity to target cells.



Track cell health of identified subsets







- Mixed populations of Jurkat (T cell lymphoma) and ThP-1 (leukemic monocyte) cell lines were added to wells containing FabFluor-488 labelled α-CD3 Ab (green) and Annexin V (red).
- Fas-ligand (10 µg/ml) which is known to induce apoptosis in T cells was also included in the wells.
- Cell count was acquired using cell-by-cell analysis, as well as morphology and fluorescence data for each single cell identified.
- Green and red intensity for all events (cells) are described on a 2D plot with gates being applied in order to create cell subsets.
- Cell death in the CD3+ subset was found to be directly proportional to the percentage of Jurkat cells added to wells.