Continuous live-cell proliferation, clustering and viability assays for T-cells, PBMCs, monocytes and B-cells

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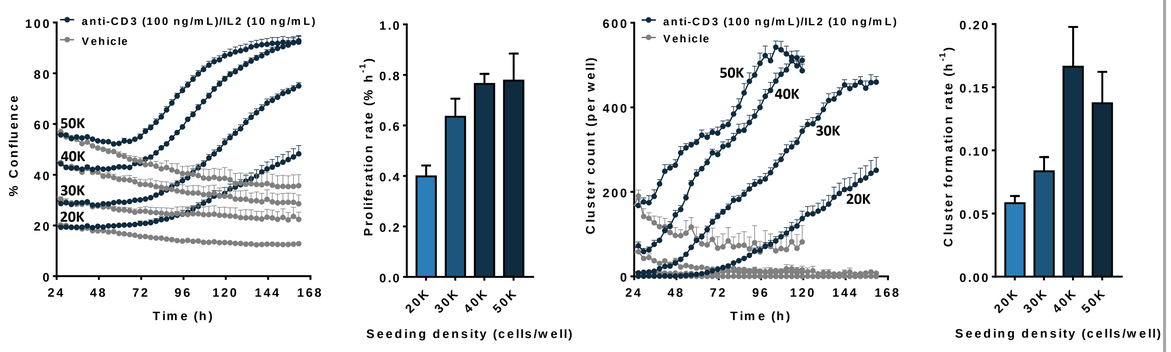
Overview

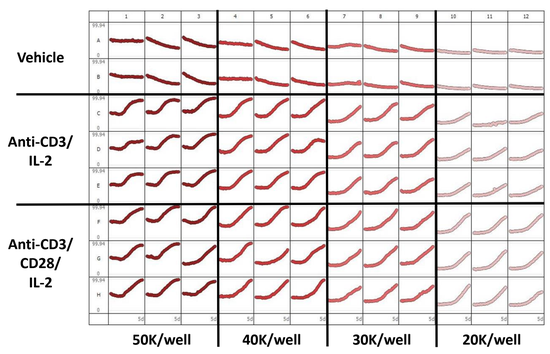
- Standard techniques for monitoring non-adherent immune cell physiology include flow cytometry, ^{3}H thymidine and ATP assays.
- These methods are perturbing to cells and do not provide additional biological insight.
- Conventional microscopy overcomes these limitations but is infrequently used as non-adherent cells can be hard to image.
- Here, we have developed and validated continuous live-cell assays for non-adherent cells using IncuCyte® ZOOM.
- The approach is amenable to all non-adherent cells and does not interfere with their inherent biology.
- The data presented here demonstrates how simple methodology can be integrated with IncuCyte ZOOM to provide a powerful technological tool for immunology researchers.

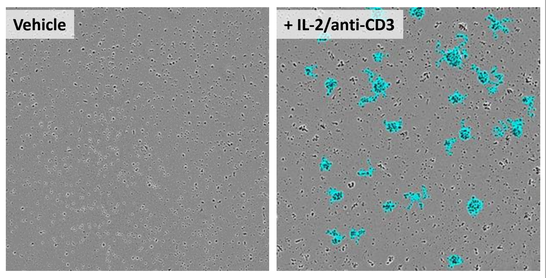
T-cell proliferation & clustering is seeding density-dependent



- T-cells demonstrate little or no proliferation under basal conditions but rapidly proliferate when activated (e.g. by IL-2, anti-CD3, anti-CD28).
- Following activation, T-cells also form cell clusters; imaging enables quantification of this phenotype.

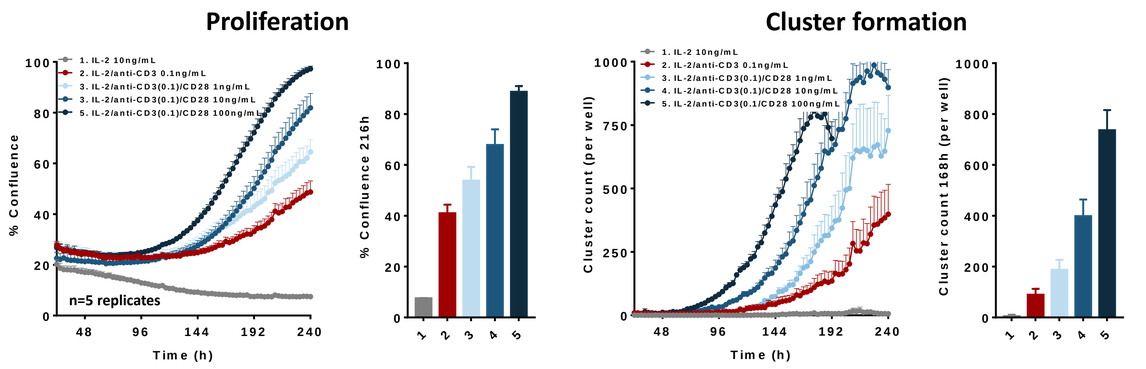
Automated 96-well continuous analysis





- The confluence of unstimulated PBMCs can be seen to drop over time due to the possible presence of phagocytes.

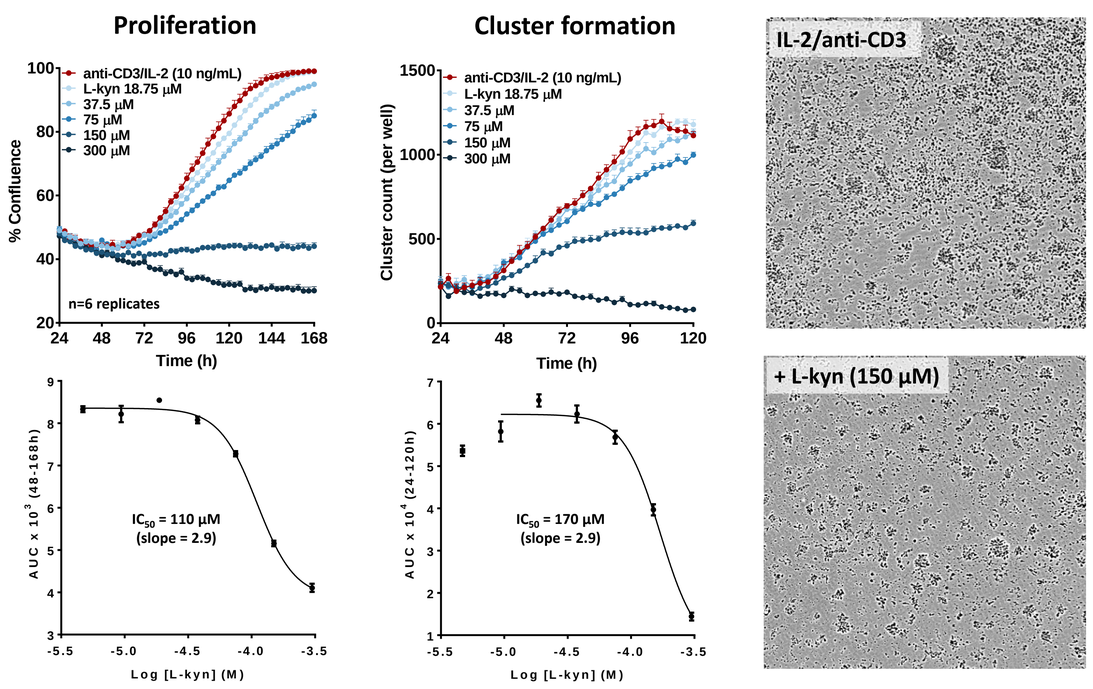
T-cell activation is stimulus and concentration-dependent



- Data shown are for PBMCs treated with combinations of IL-2, anti-CD3, and anti-CD28.

L-kynurenine inhibits T-cell proliferation and clustering

- L-kynurenine (L-kyn) is a metabolite formed from the catabolism of L-tryptophan by the enzymes IDO and TDO.
- Some cancers increase L-kyn production in a bid to block antigen-driven T-cell proliferation and induce T-cell death, thus allowing cancer cells to escape immune surveillance.
- Inhibitors of IDO and/or TDO are therefore promising therapeutic targets for the treatment of cancer.

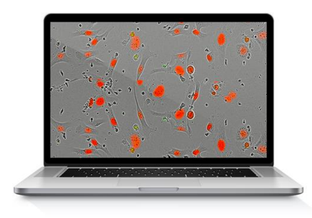


- Data demonstrates a clear concentration-related inhibition of IL-2/anti-CD3 activated T-cell proliferation and clustering with the addition of exogenous L-kyn, over time.
- Time-course profiles enabled AUC analysis and generation of concentration-response curves from which IC_{50} values for inhibition of proliferation and clustering were determined.

Continuous Live Cell Analysis: Methodology

IncuCyte® ZOOM System

A fully automated phase-contrast and 2-colour fluorescence imager that resides within a standard cell incubator for optimal cell viability. Designed to scan plates & flasks repeatedly over time.



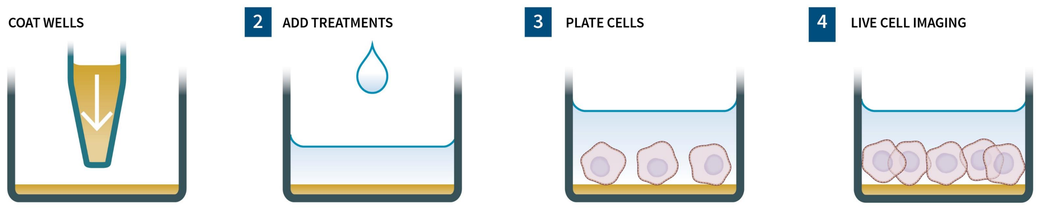
IncuCyte® Software

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

IncuCyte® Reagents & Consumables

A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted GFP & RFPs for cell counting, no-wash caspase 3/7 substrate for apoptosis and cell kits for angiogenesis.

Non-adherent cell assay methodology



Add plate coating
Coat wells of plate (50 µL/well) with your choice of plate coating.

Add test agents
Add desired treatments (100 µL/well) at 2X final assay concentration.

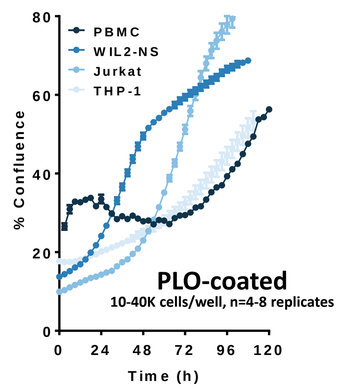
Seed cells
Seed cells (100 µL/well, 5,000-50,000 cells/well) into the 96-well plate).

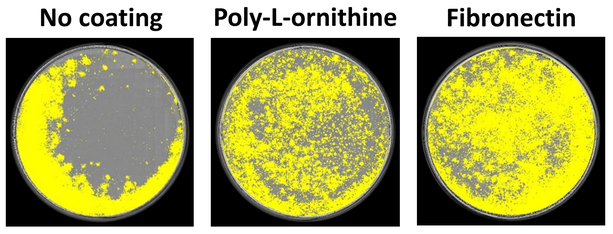
Automated imaging and quantitative analysis
Capture images every 1 to 4 hours (10X or 4X) in an IncuCyte® ZOOM system. Analyze using integrated software.

Plate-coatings enable cells to remain in the field-of-view

Cell type	Description	Poly-L-Ornithine	Fibronectin	Laminin	Matrigel ™	Poly-D-Lysine	FBS	Gelatin	Cell-Tak™	PureCol®
Jurkat	T lymphoma	✓	✓	×	×	✓	×	×	✓	×
WIL2-NS	B lymphoma	✓	✓	×	×	✓	×	×	✓	×
RAMOS	B lymphoma	✓	✓	×	×	NT	NT	NT	NT	NT
THP-1	Monocytic	✓	✓	✓	×	✓	×	×	✓	×
PBMCs	Human Primary	✓	×	×	×	×	×	×	×	×
T-cells	Human Primary	✓	×	×	×	NT	NT	NT	NT	NT

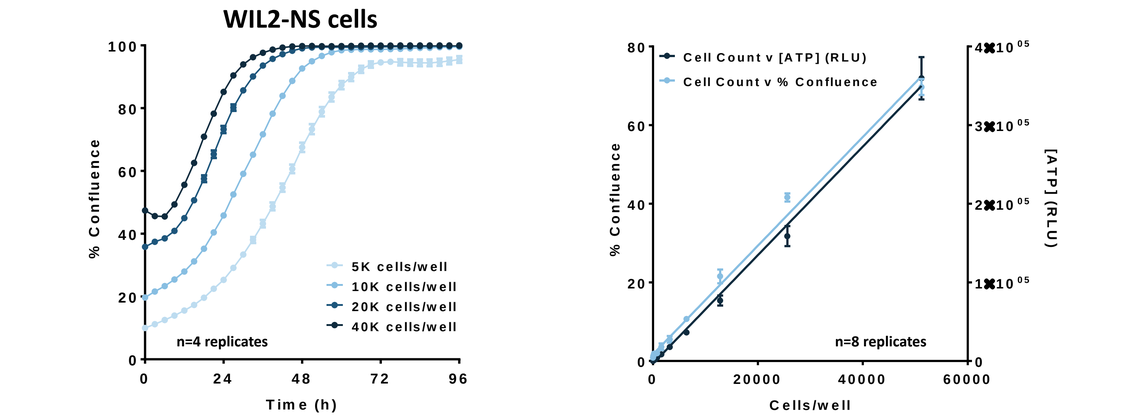
NT = not tested
NB = PureCol® provides an even cell coverage but is fibrous and therefore unsuitable for imaging





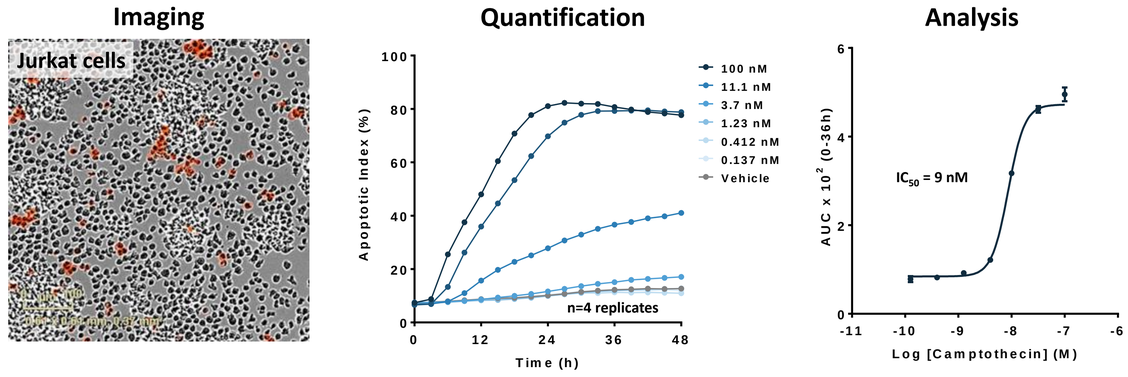
- Poly-L-ornithine performs better than other coatings in supporting proliferation and providing a uniform cell distribution for imaging. Fibronectin is also suitable for most cell types but is known to induce cell proliferation.

Confluence is a validated measure of cell number



- Non-adherent cell proliferation is quantifiable with IncuCyte ZOOM and fully-validated against direct cell counting and ATP measurement.
- WIL2-NS cells counted using a Scepter™ and seeded at various densities onto PLO-coated 96-well plates.
- Cell number quantified using phase contrast imaging (IncuCyte ZOOM) or ATP luminescence assay.

Phase-contrast can be duplexed with cell health reagents



- Phase-contrast analysis can be duplexed with cell health reagents (e.g. IncuCyte Cytotox Reagents) and/or apoptosis markers (IncuCyte Caspase-3/7 or Annexin V Reagents).
- Shown here is data generated with Jurkat cells treated with the topoisomerase inhibitor, camptothecin.
- Concentration-response curves were generated from time-course profiles to enable determination of IC_{50} values.