# Quantitative Live-Cell Imaging Assays for Immmunotherapy: Chemotaxis, Immune Cell Killing and Phagocytosis

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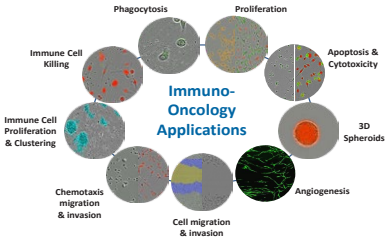


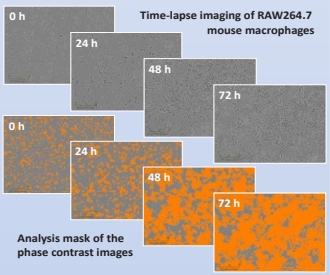

## Summary and Impact


- For the body to defend and fight against cancer, immune cells must recognise, engage, destroy and ultimately remove unwanted tumour cells. Understanding these processes and interactions at the cellular level is central to identifying and validating new drug targets and cellular therapy approaches.
- Essen BioScience offers a flexible range of phenotypic assays to explore all aspects of the immuno-oncology research area. All of these assays are based on non-invasive live-cell analysis of cells in 96-well micro-plates using an IncuCyte® ZOOM live cell analysis system.
- Here we describe a cluster of new assays for quantifying immune cell biology and interactions with tumour cells.
- Phase-contrast/fluorescence images from cells gathered over time are processed to measure phenotypes, such as immune cell proliferation, migration & death.
- Experiments are performed with low cell numbers using simple mix and read formats which are non-perturbing to the cell model.
- Each of these approaches provide a full time-course of the biology; images and time-lapse movies provide credibility and valuable biological insight. Added throughput and automated image analysis enhances productivity.


## Workflow
### Micro-titre Plate Models

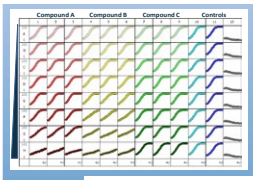
- Live cell protocols enable real time measures within your incubator - novel instrumentation, reagents & consumables.
- Simple mix-and-read methods - no wash, no fix, no cell lifting.
- Analyse up to 6 plates in the IncuCyte® system.

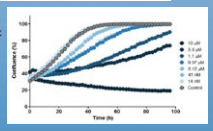
### Automated Imaging and Analysis

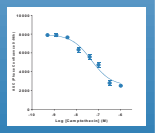



- Time-lapse images taken from every well and automatically analysed.
- Non-perturbing, non-invasive and direct measures of phenotypic cell biology.
- High definition phase contrast image processing and fluorescence object quantification.

### Real Time Quantification



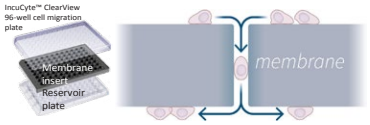




Quickly visualise time-course data and quantify phenotypic biology.

- Flexible graphing and data export capabilities.
- Information rich images and movies.

## Chemotaxis

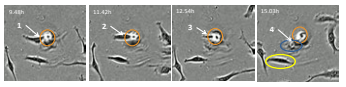



**PRINCIPLE: Cells seeded onto the top surface of the IncuCyte™ ClearView membrane insert and migrate towards chemoattractant in the lower reservoir plate.**

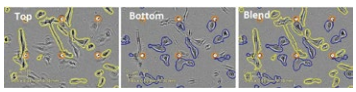
Cell movement monitored over time, imaging every 0.5-2 h. IncuCyte® ZOOM live cell analysis system images and quantifies the top and bottom surface of the membrane. Cell movement is detected as a loss of cell area from the top surface or an increase in cell area on the bottom surface .

**APPLICATIONS: Measure migration, invasion or trans-cellular migration (e.g. TEM) with both adherent and non-adherent cells.**

### Visualise and Quantify Images

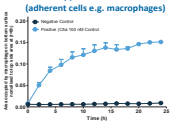


1 & 2. Images of a cell migrating towards a pore (orange) in response to chemoattractant.
3 & 4. Cell migrates through the pore and then appears on the underside of the membrane.



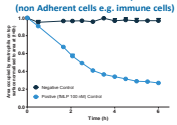

Automated image processing separates cells located on the top surface (yellow) and bottom surface (blue) of the membrane, pores shown (orange). Images are processed at acquisition.

**Measure Cell Appearance On Bottom Surface (adherent cells e.g. macrophages)**



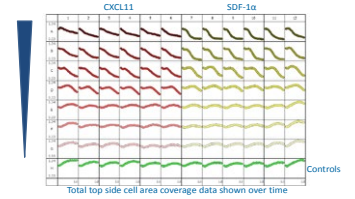
- Data shows response of HBDM to C5a.
- Adherent cells migrate across the membrane, through the pores and then remain attached to the bottom surface of the membrane.

**Measure Cell Loss From Top Surface (non Adherent cells e.g. immune cells)**

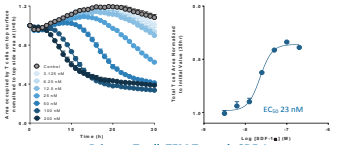


- Data shows response of neutrophils to fMLP.
- Non-adherent cells migrate across the membrane, through the pores and drop into the reservoir plate.

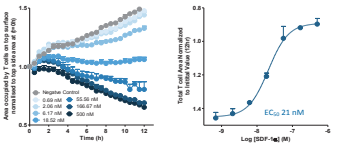
### Primary T Cell Pharmacology




**Chemotaxis of Primary T Cells to SDF-1α**

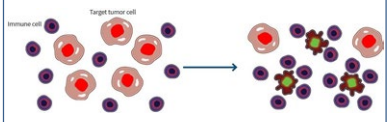



**Primary T cell, TEM Towards SDF-1α**




- Data shows CD3/CD28 activated T cell migration over time towards SDF-1α as both chemotaxis and extravasation through a HUVEC monolayer.
- The data shows there was a concentration-dependent loss of cell area from the top surface in both assay formats.

## Immune Cell Killing

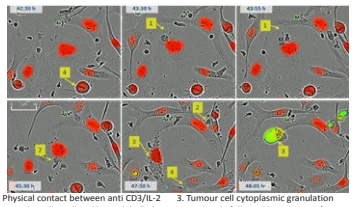



**PRINCIPLE: Target cells (RFP-nuclear labelled) co-cultured with immune cells (T cells, NK, PBMC), with various activators and the IncuCyte® ZOOM Caspase 3/7 apoptosis reagent (green).**

Cells monitored over time, imaging every 2 h. IncuCyte® ZOOM live cell analysis system images and quantifies phase and fluorescence images Target cell number is quantified as the number of red objects (nuclei), apoptosis by counting the green-labelled nuclei.

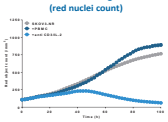
**APPLICATIONS: Measure T-cell killing/ADCC in adherent or non adherent target cells.**

### Visualise and Quantify Images



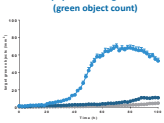
1. Physical contact between anti CD3/IL-2 activated small T cell and larger labelled SKOV3 NucLight Red™ cell. T cell division.
2. Tumour cells under attack from T cells.
3. Tumour cell cytoplasmic granulation immediately followed by caspase 3/7 labelling, nuclear condensation & cell death.
4. Tumour cell mitosis: one cell becomes two.

**Proliferation of target cells (red nuclei count)**



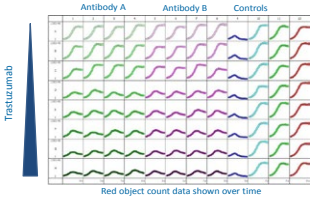
- No effect on target cell growth in the presence of non-activated PBMCs.
- Addition of anti-CD3/IL-2 yields marked attenuation of target cells numbers.

**Apoptosis of target cells (green object count)**

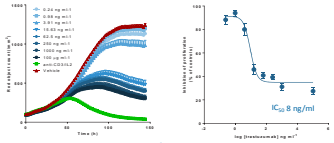


- In the presence of anti-CD3/IL-2 activated PBMCs there is a marked increase in green objects over time representing apoptotic target cells.

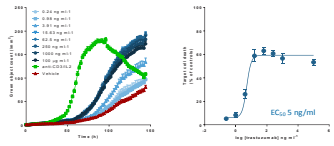
### Antibody-Dependent Cell Cytotoxicity




**Proliferation of Target SKOV3 Cells**

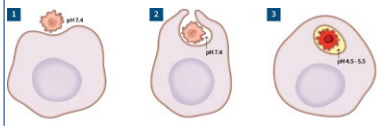



**Apoptosis of Target SKOV3 Cells**




- Her2 positive SKOV3 NucLight Red™ cells combined with PBMCs were tested in the presence of Trastuzumab to induce antibody-dependent cell-mediated cytotoxicity (ADCC).
- Concentration-dependent decrease in proliferation and increase in apoptosis.
- No response was seen in Her2 negative cells (A549; lung carcinoma).

## Phagocytosis

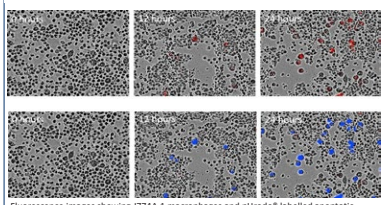



**PRINCIPLE: pHrodo® labelled cells added to phagocytes (e.g. macrophages), phagocytosis is initiated following receptor activation and pHrodo® labelled cells are engulfed.**

Cells monitored over time, imaging every 15-30 min. Little or no fluorescence is measured while target cells are in the extracellular environment (pH 7.4). Once in the acidic phagosome (pH 4.5-5.5) there is an increase in pHrodo® fluorescence.

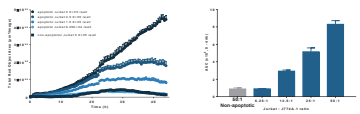
**APPLICATIONS: Measure phagocytosis, efferocytosis and ADCP of pHrodo® labelled target cells.**

### Visualise and Quantify Images



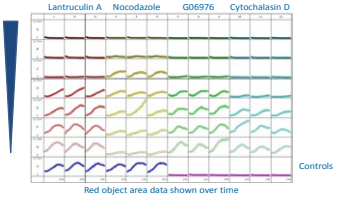

Fluorescence images showing J774A.1 macrophages and pHrodo® labelled apoptotic Jurkat cells (top row).
Masking of red fluorescent objects (blue) for analysis and enabling quantification (bottom row).

**Phagocytosis of apoptotic pHrodo® labelled Jurkat cells (efferocytosis)**

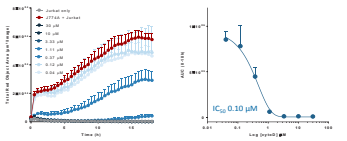


- Addition of apoptotic cells to the J744A.1 macrophages results in an increase in fluorescent area as labelled cells transition into the acidic phagosome .
- Amplitude of fluorescent signal is proportional to the number of target cells added.
- The fluorescent signal is minimal with the addition of non-apoptotic Jurkat cells.

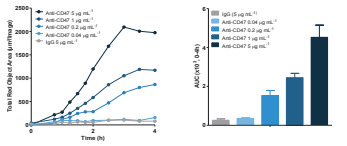
### Modulation of Phagocytosis




**Inhibition of Phagocytosis by Cytochalasin D**




**Enhancement of Phagocytosis by Anti-CD47 Antibody**



- Inhibition data; efferocytosis of apoptotic Jurkats by J744A.1 macrophages.
- Enhancement data; phagocytosis of CCRF-CEM cells in the presence of increasing concentrations of anti-CD47 by BMDM.
- Inclusion of anti-CD47 binds to the "don't eat me" signal on CCRF-CEM to promote phagocytosis by the macrophages.

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