Real time image-based quantification of phagocytosis in living cells using IncuCyte ZOOM®

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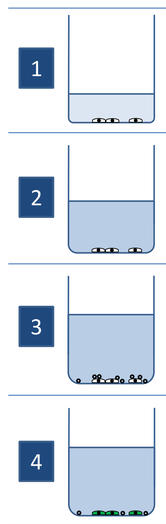
Summary & Impact

- Phagocytosis is a specific form of endocytosis by which cells internalize solid matter, thus eliminating cellular debris and pathogens. This process is predominantly performed by the phagocytes of the immune system (macrophages, neutrophils and immature dendritic cells).
- We have developed a new image-based methodology that combines IncuCyte® pHrodo® Bioparticles® and image-based fluorescent measurements, enabling simple mix and read protocols suitable for screening.
- The approach is amenable to many phagocytes and evaluation of the effects on phagocytosis of small molecules, biologics and gene-interference reagents (e.g. siRNA, miRNA).
- Temporal differences were observed when studying the engulfment of IncuCyte® pHrodo® Bioparticles® derived from different micro-organisms.
- Kinetic analysis reveals temporal differences in the profile of different pharmacological agents.
- This model enables real-time visualisation and quantification of phagocytosis, with the potential to better model the dynamics of the process.

Fully integrated real-time phagocytosis assay



1 Seed cells

Seed phagocytic cells (e.g. J774A.1, 1-10K/well) in 96-well plates. Culture overnight (50 µl/well).

2 Apply treatments

Treat cells with compounds prior to phagocytosis (0.5-24 h pre-treatment, 25 µl/well).

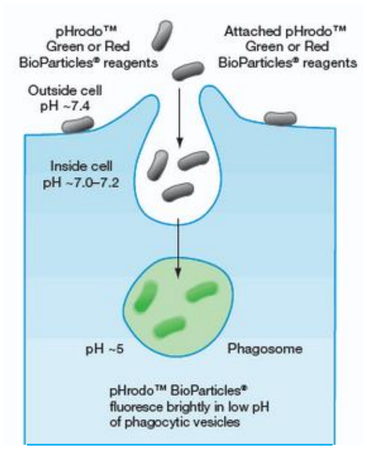
3 Add IncuCyte® pHrodo® Bioparticles®

Add sterile IncuCyte® pHrodo® BioParticles® to treated wells (10 µg/well, 25 µl/well).

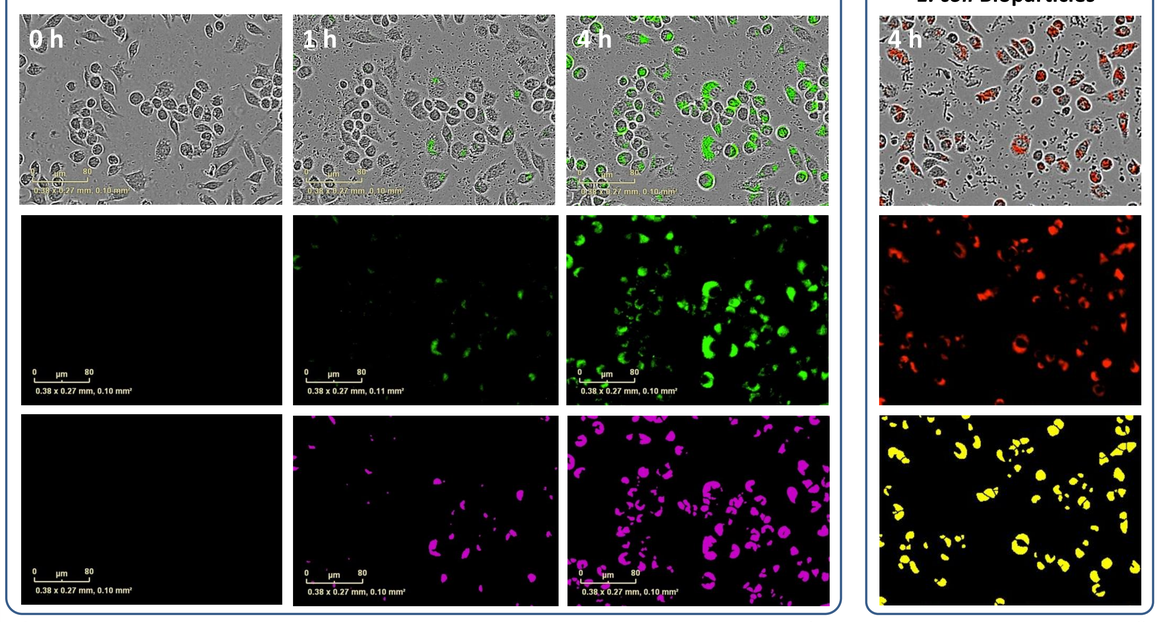
4 Live Cell Fluorescent imaging

Capture images every 10-30 mins (20x or 10x) in IncuCyte ZOOM® for 2 - 48 hours, analyse using integrated software

pH-sensitive Bioparticles® fluoresce in the acidic environment of the phagosome

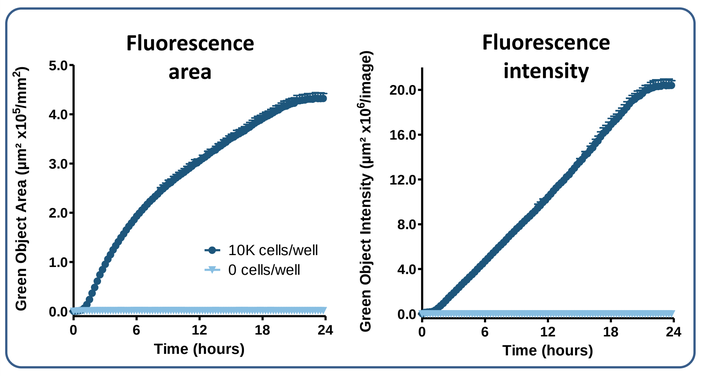


Real-time visualisation of phagocytosis

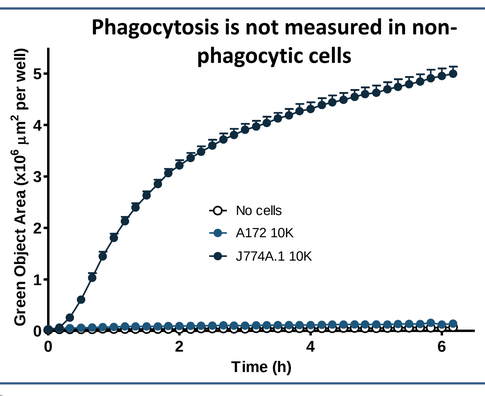


- Representative images of a J774A.1 mouse macrophages (seeded at 10K cells/well) in the presence of IncuCyte® pHrodo® Green *E. coli* Bioparticles® (10 µg/well, left panel) or IncuCyte® pHrodo® Red *E. coli* Bioparticles® (10 µg/well, right panel).
- Blended images of phase contrast and green/red fluorescent images (top row), fluorescent images alone (middle row) or the segmentation mask derived from the fluorescent images (bottom row) (20x or 10x magnification).

Quantification of phagocytosis

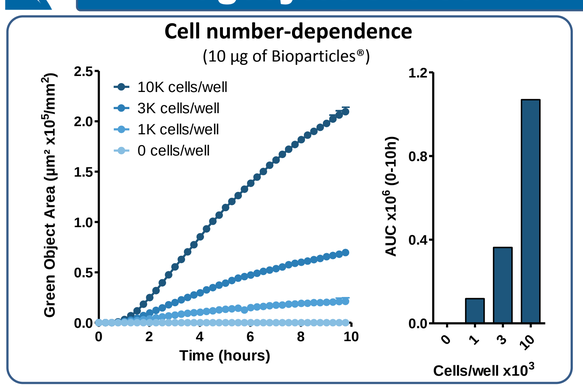


- **Fluorescence area**; surrogate for phagosome counting. Measured as area of masked area. Note cell proliferation contributes to increase during the time-course.
- **Fluorescence intensity**; surrogate for internalisation into and maturation of phagosomes. Fluorescence intensity continues to increase as the pH within the phagosome lowers during the maturation to phagolysosomes.

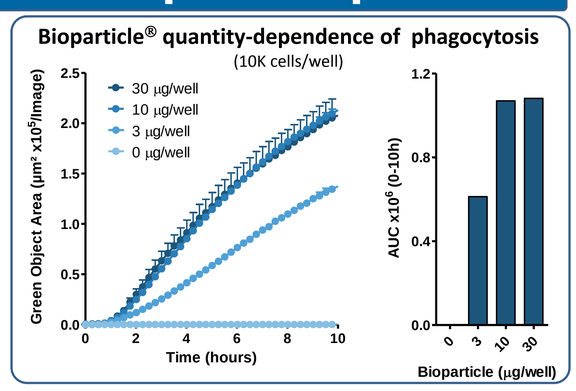


- Non-phagocytic cells (A172; brain glioblastoma), seeded at 10K cells/well, yielded little or no increase in fluorescence when exposed to IncuCyte® pHrodo® Green *E. coli* Bioparticles® (10 µg/well).
- In the same experiment substantial fluorescence was observed with J774A.1 mouse macrophages, in the presesence of IncuCyte® pHrodo® Green *E. coli* Bioparticles® (10 µg/well).

Phagocytosis is cell number- & bioparticle-dependent

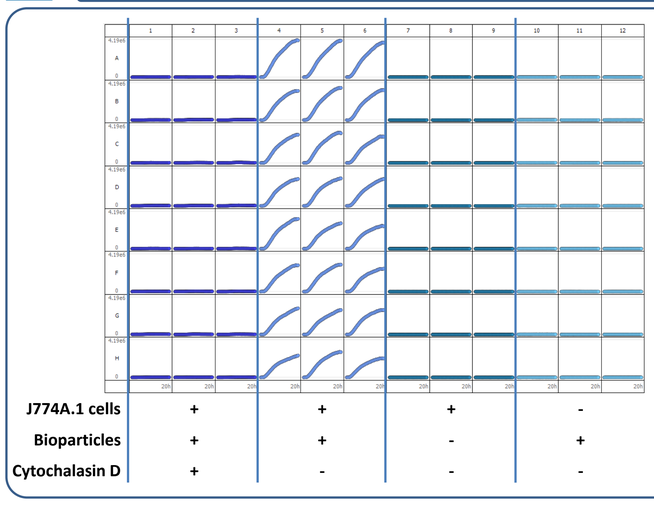


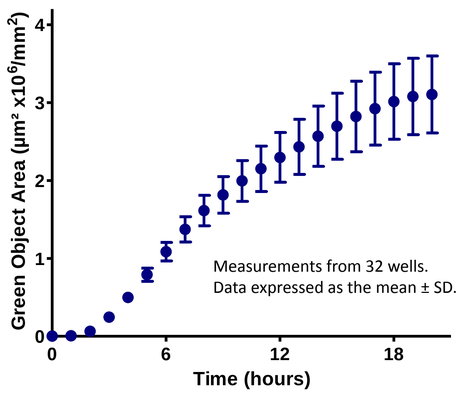
- Phagocytosis of IncuCyte® pHrodo® Green *S. aureus* Bioparticles®, as measured by the increase in fluorescence area, is proportional to the number of cells.
- Note the small error bars even when 1000 cells per well are used, indicative of low cell usage.



- Increased phagocytosis is observed when the quantity of bioparticles is increased.
- The process appears saturated at high concentrations (>10 µg per well, 10K cells per well).

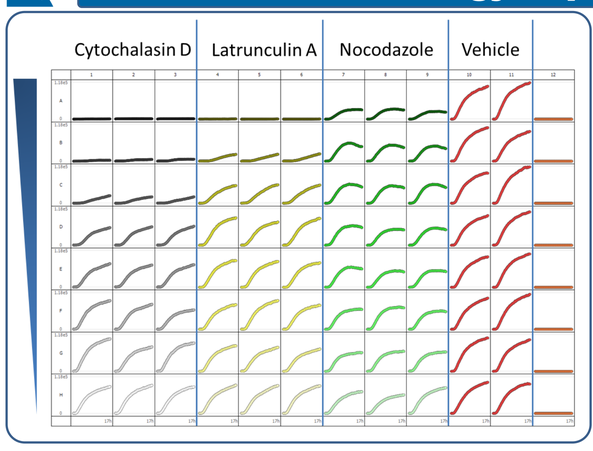
Highly robust phagocytic measurements





- **Robust fluorescence signal**; substantial, time-dependent increases in fluorescence were observed in wells containing J774A.1 cells and IncuCyte® pHrodo® Green *E. coli* Bioparticles®.
- **Minimal background signal**; little or no fluorescence was observed when Cytochalasin D (30 µM) was added to the J774A.1/bioparticles and when cells or bioparticles were added alone.

Pharmacology of phagocytosis inhibitors



- Compounds were added to J774A.1 macrophages 1 hour prior to addition of IncuCyte® pHrodo® Green *E. coli* Bioparticles® (10 µg/well).
- Increase in fluorescence area and intensity was measured over time (area data shown).
- **Cytochalasin D** (actin polymerisation inhibitor) and **LY294002** (PI3K inhibitor) inhibit phagocytosis in a concentration-dependent, but not time-dependent manner.
- **Nocodazole** (microtubule formation disruptor) shows limited concentration-dependence and appears to exert a greater effect at later time-points.