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Simplifying Progress

Use of Fluorescent Fab/Ab Complexes and Incucyte® Live-Cell Analysis to Dynamically Track Cell Surface Markers and Cell Populations in Mixed Cultures

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Summary and Impact

- Fluorescently-labeled antibodies are widely used for visualizing cell-surface protein expression and immunophenotyping (e.g. immunocytochemistry and flow cytometry).
- However, their applications are largely confined to endpoint or short-term (min–hr) detection, and the cell processing and labeling steps that are required often perturb the biology of interest.
- To enable longer-term, fully dynamic applications of cell surface protein markers in living cells, we have developed a novel strategy based on fluorescently-labeled antibody fragments (Fabs) and Incucyte® live-cell analysis.
- An anti-mouse Fc-targeted Fab fragment conjugated to a green fluoroprobe (Incucyte® Fabfluor-488 Antibody Labeling Dye) was used to tag Abs to surface markers (e.g. CD4, CD20) via a simple, one-step no-wash protocol.
- Addition of the Fabfluor-488-Ab complex to living cells produces a long-lasting, specific and stable fluorescence and does not perturb cell morphology or growth.
- This methodology enables long-term tracking and quantification of protein expression and the ability to identify cell subsets in living cultures over time.
- This approach should prove powerful in analysis on complex and advanced heterogeneous cell models.

Incucyte® Live-Cell Imaging and Analysis: Methodology

Incucyte® Live-Cell Analysis System

A flexible assay platform that sits inside a standard tissue culture incubator. Incucyte® automatically and continuously acquires and analyzes HD phase-contrast and fluorescent images of living cells cultured in microplates, dishes, or flasks.

Incucyte® Software

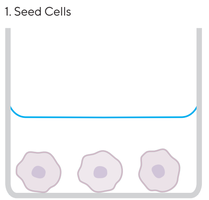
Fast, flexible, and powerful control hub for continuous live-cell analysis comprising image acquisition, processing, and date visualization.

Incucyte® Reagents and Consumables

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

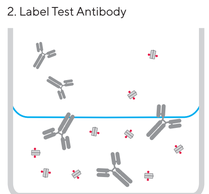
Quick Guide

1. Seed Cells



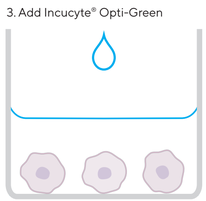
Cell Seeding
Seed cells (50 µL/well, 5–30K/well) into 96-well plate.
Note: For non-adherent cell types, PLO coat plate prior to cell seeding.

2. Label Test Antibody



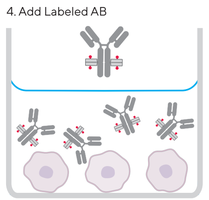
Labeling of Test Antibody with Incucyte® Fabfluor-488 Dye
Mix antibody and Fabfluor Dye at a molar ratio of 1:3 in media, 2X final assay concentration. Incubate for 15 minutes to allow conjugation.

3. Add Incucyte® Opti-Green



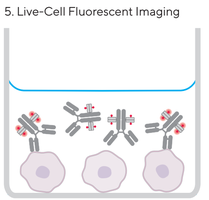
Incucyte® Opti-Green Background Suppressor Addition
Add 50 µL/well, (3X final concentration).

4. Add Labeled AB



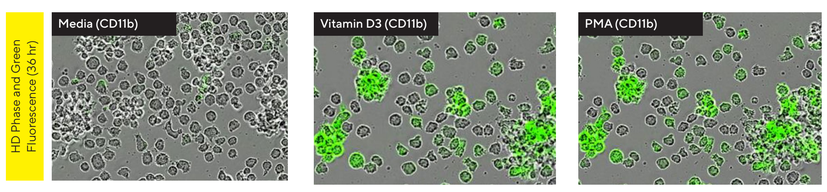
Incucyte® Fabfluor-488-Labeled Antibody Addition
Add antibody-Fabfluor mix (50 µL/well) to cell plate. Non-adherent cells—spin plate.

5. Live-Cell Fluorescent Imaging

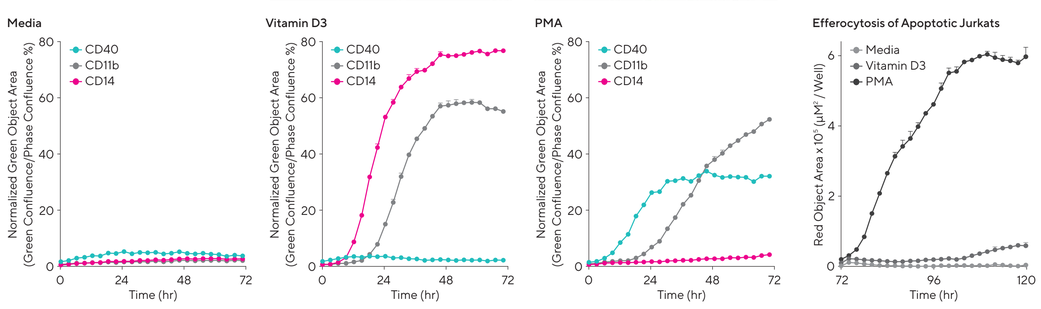


Automated Imaging and Quantitative Analysis
Capture images every 15–30 minutes (10X or 20X) in Incucyte® Live-Cell Analysis System for 24–48 hours. Analyze using integrated software.

Coupling Protein Expression Dynamics to Cell Differentiation



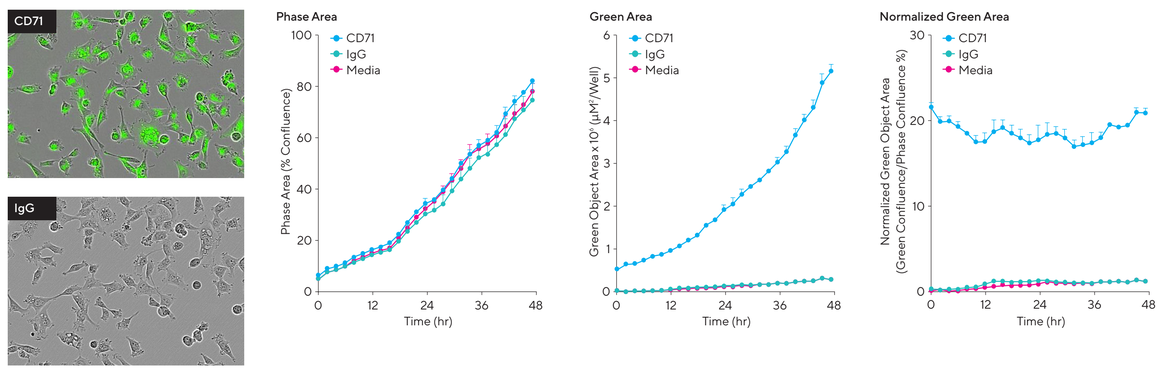
- THP-1 cells (30K/well) were incubated with Incucyte® Opti-Green, Fabfluor-488-Ab and either Vitamin D3 (50 nM) or phorbol myristate acetate (PMA; 100 nM) to induce a macrophage-like phenotype.
- Phase and fluorescence images were captured (20X) objective).



- THP-1 cells treated with Vitamin D3 or PMA show differences in morphology, protein expression and function (efferocytosis).
- Both treatments enhance CD11b expression but only Vitamin D3 upregulates CD14, while only PMA induces CD40.
- PMA-treated THP-1 cells exhibit a macrophage-like morphology, proliferate at a slower rate, and display efferocytic potential.

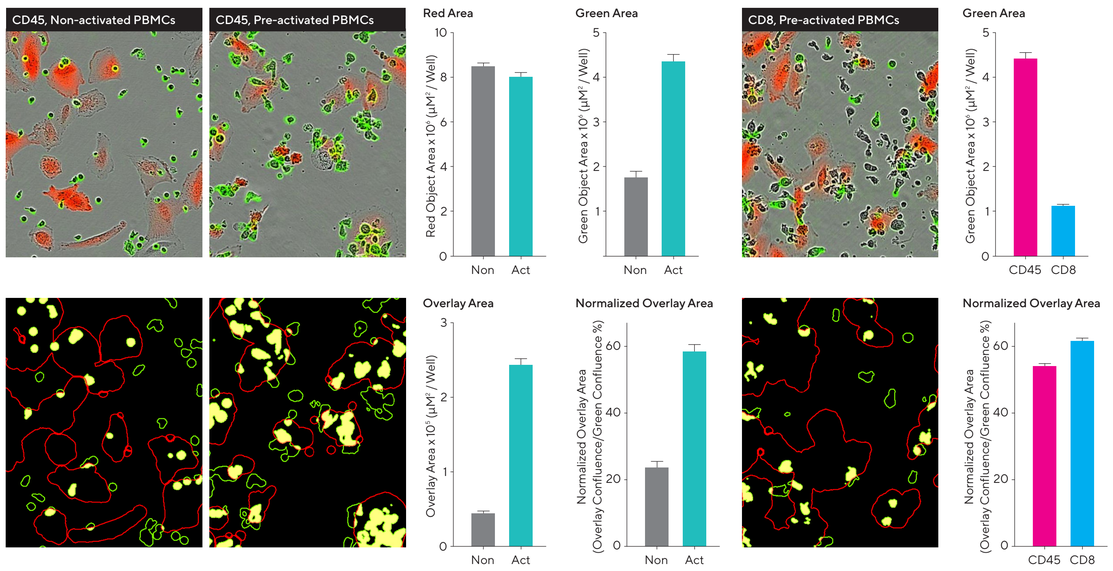
Validation: Non-Perturbing, Sustained, and Specific Labeling

- HT-1080 fibrosarcoma cells (5K/well) were incubated with Incucyte® Opti-Green Background Suppressor (0.5 mM) in the presence of media or antibodies to CD71 (transferrin receptor) and IgG (isotype control) labeled with Fabfluor-488 reagent.



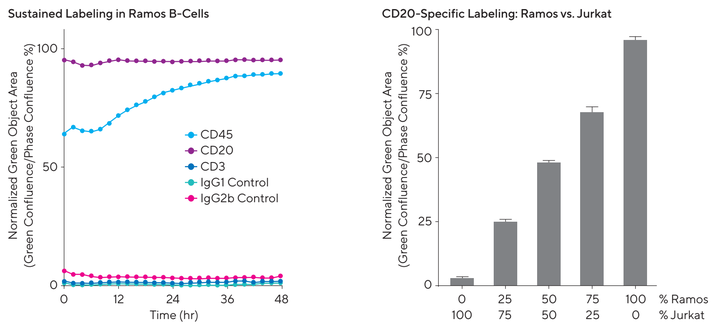
- CD71 labels the entire HT-1080 cell population. No cell labeling was observed with IgG.
- CD71 Fabfluor-488-Ab complex in the presence of Incucyte® Opti-Green does not impair cell proliferation (Phase Data).
- CD71 expression increases over time, in line with cell proliferation.
- Normalized green/phase area metric confirms the stability and longevity of CD71 labeling.

Monitoring Cell-to-Cell Interactions: Immune Cell Killing



- Incucyte® Cytolight Red labeled A549 Cells (adenocarcinoma) (5K/well) were incubated with pre-activated PBMCs (anti-CD3/IL-2, 4 day) in the presence of Incucyte® Opti-Green and CD 45 or CD8 Abs labeled with Incucyte® Fabfluor-488.
- Images were captured 2 hr post-set-up (20X objective).
- Pre-activated PBMCs (CD45-positive), show a greater level of interaction (overlay area with A549 target cells than non-activated PBMCs. Normalized overlay area suggests that CD8-positive cells may not interact more with A549 cells than the general CD45-positive PBMC population.

Normalized Green Area

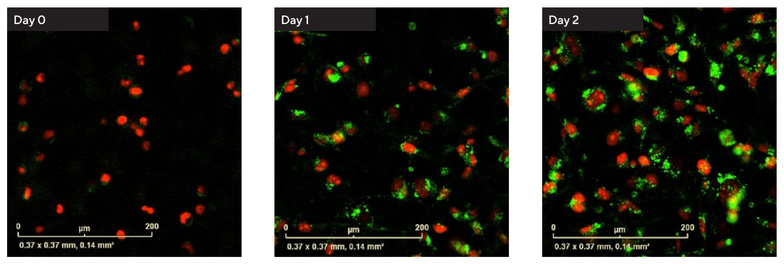


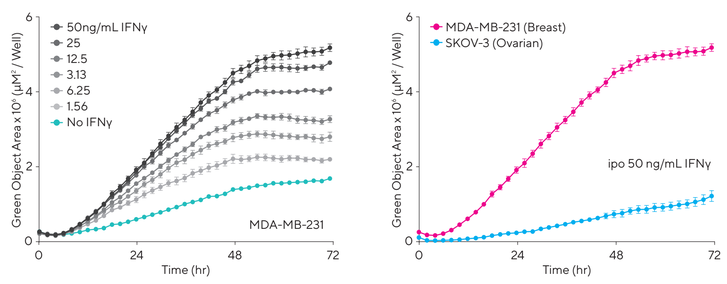
- Ramos (B-lymphoma) cell labeling with CD45 (common lymphocyte antigen), CD20 (B-lymphocyte antigen), but not CD3 Ab complex (T cell co-receptor).
- CD20 labeling area follows anticipated proportions in mixed cultures (Jurkat T cells are CD20 negative).

IFNγ-Induced Upregulation of PD-L1 Checkpoint Protein

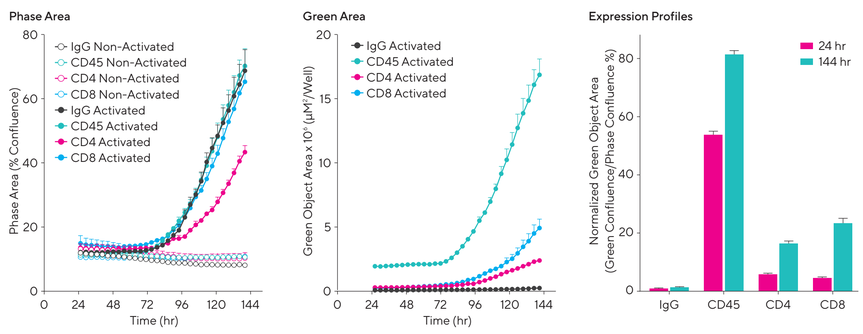
- MDA-MB-231 (breast) or SKOV-3 (ovarian) cancer cells (5K/well) were incubated with IFNγ in the presence of Incucyte® Opti-Green and Fabfluor-488-PD-L1 Ab.
- Quantification of the green fluorescence area shows that IFNγ induces a time- and concentration-dependent increase in PD-L1 expression in MDA-MB-231 cells.
- Differential PD-L1 expression in MDA-MB-231 (high expresser) and SKOV-3 (medium expresser) cells can be visualized using this methodology.

Incucyte® Nuclight Red MDA-MB-231 Cells Treated with IFNγ





Live-Cell Immunocytochemistry: PBMC Immuno-Phenotyping



- PBMCs (30K/well) were incubated with or without anti-CD3/IL-2 (10 ng/mL), in the presence of Incucyte® Opti-Green and various Fabfluor-488-Abs.
- IgG, CD45 and CD8, had no effect on PBMC proliferation. The CD4 Ab produced a significant reduction.
- Green fluorescence area provides an index of the increase in the specific subpopulations—CD45, CD8 and CD4 all increased.
- Normalized green area estimates the expression profiles of individual cell sub-populations. The true expression profile is underestimated as the green area is smaller than the phase area within each cell.