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Quantifying cell subsets and heterogeneity in living cultures using real-time live-cell analysis
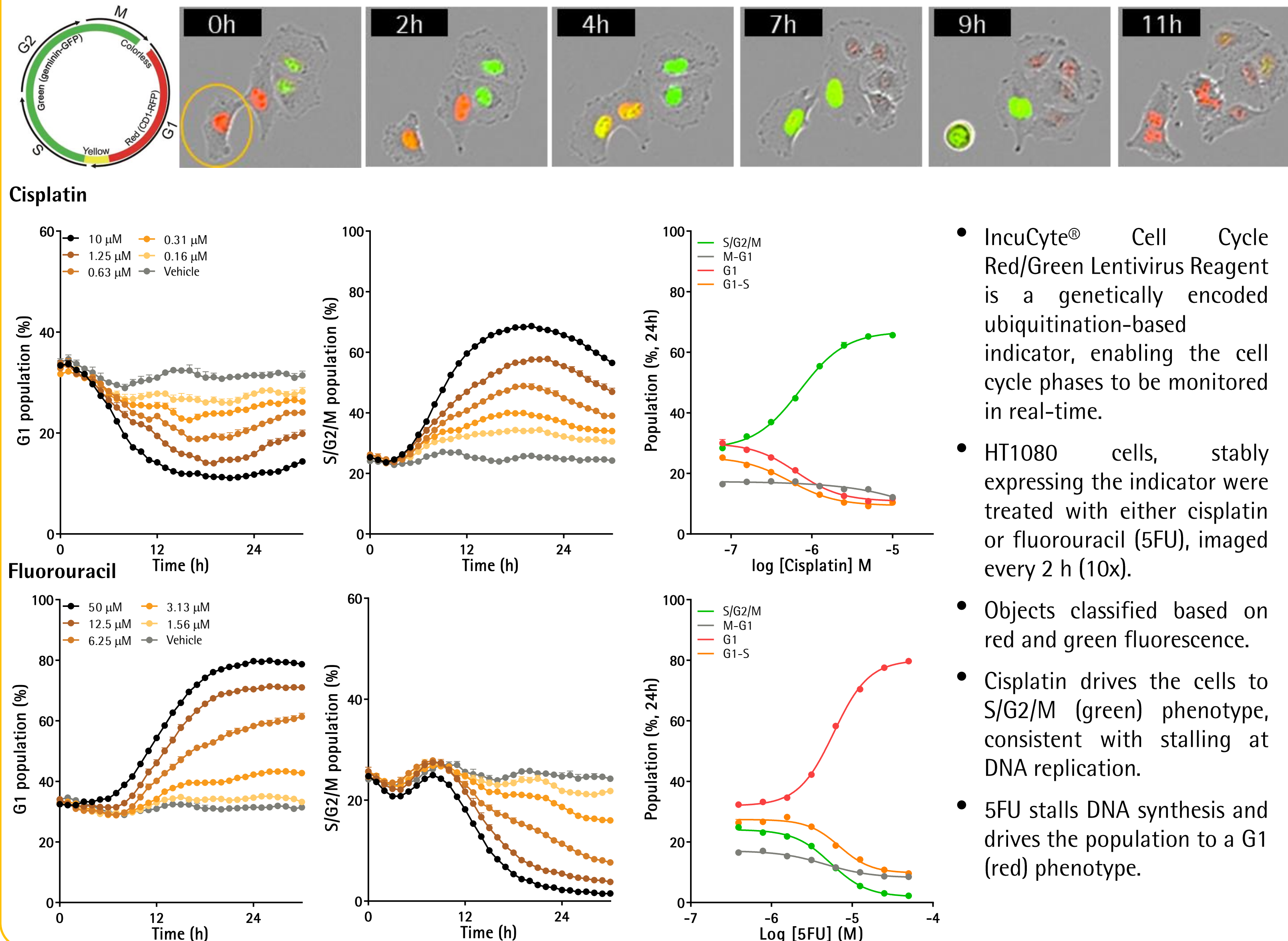
N. Bevan, T. Jackson, C. Szybut, L. Kelsey, H. Campwala, T. Dale, N. Dana, N. Holtz, E. Endsley, D. Appledorn, C. Schramm, L. Skerlos, R. Lister, D. Trezise

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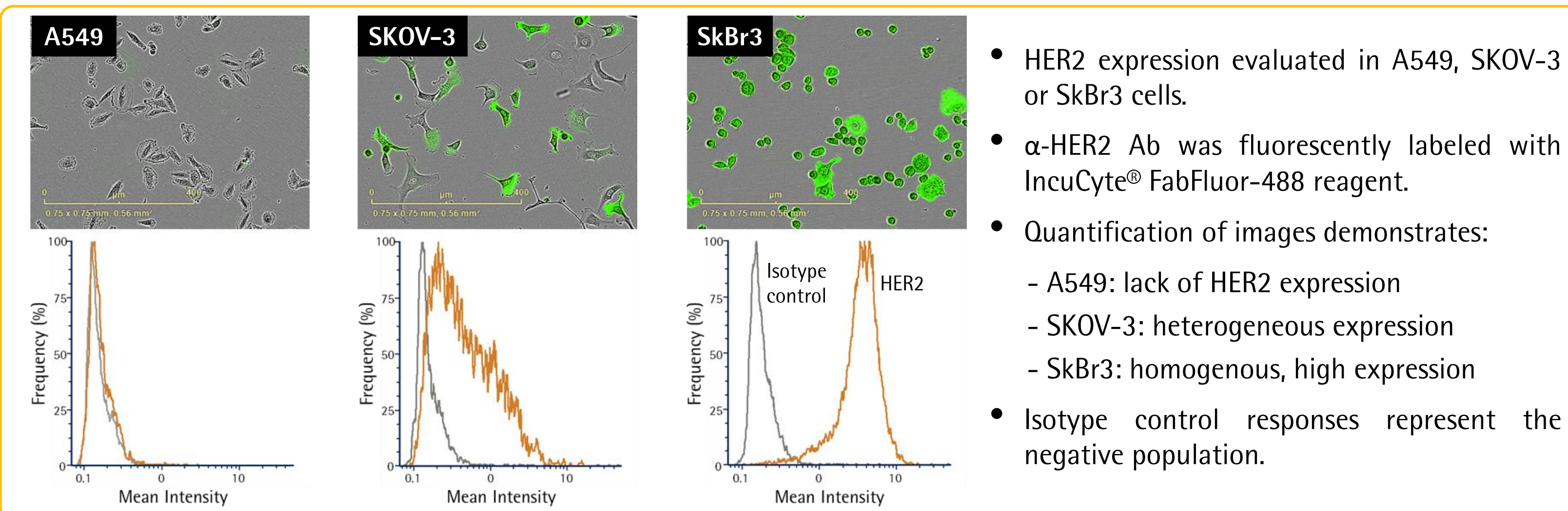
Summary & Impact

- 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 Analysis software module and associated reagents provide 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 IncuCyte® Cell-by-Cell Analysis segmentation & classification with various examples of biological activity to show parameter changes over time. Importantly, these changes can be related to function.
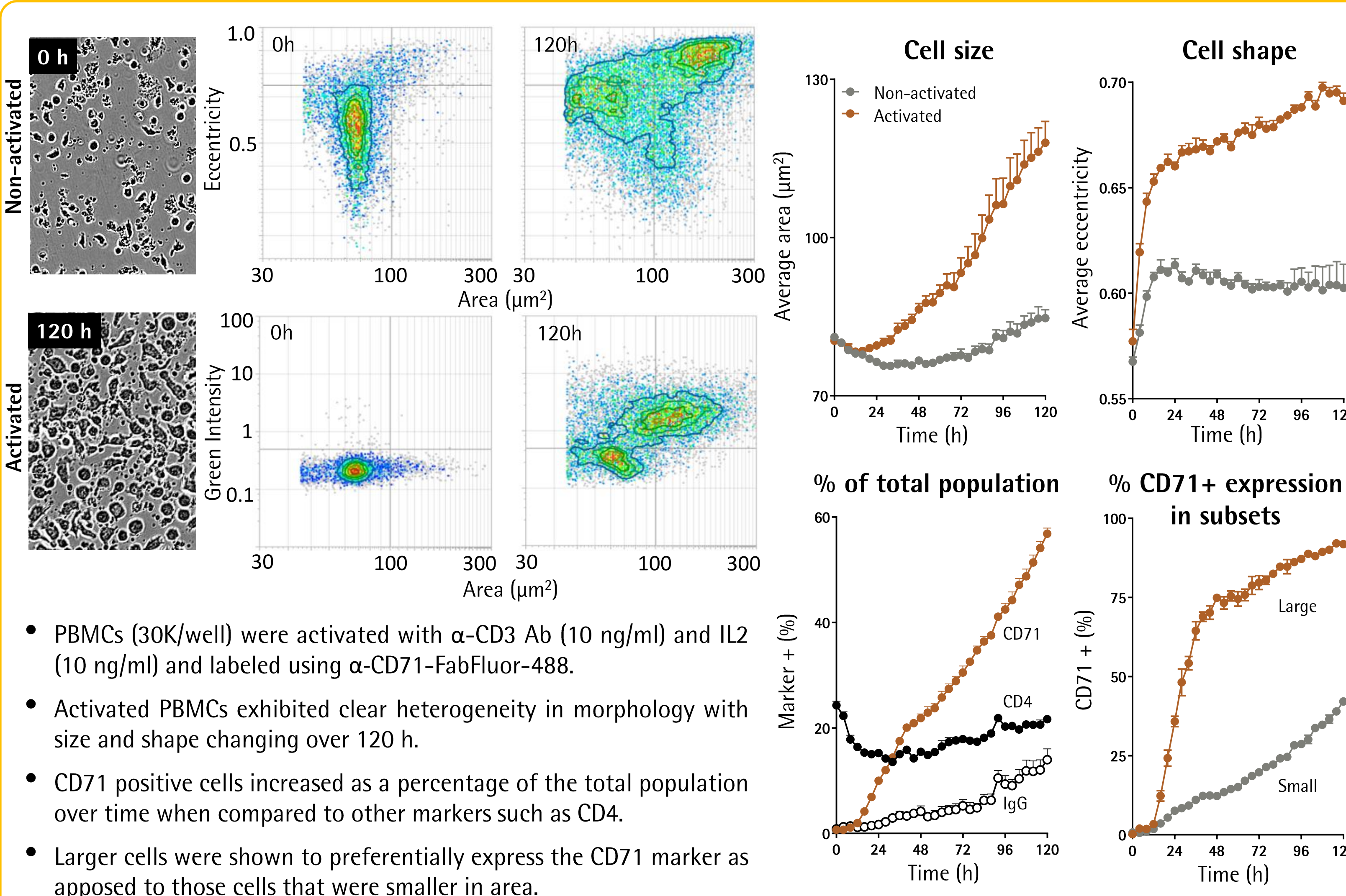
Analysis of cell cycle using live-cell analysis

Visualize phases of cell cycle over time

- IncuCyte® Cell Cycle Red/Green Lentivirus Reagent is a genetically encoded ubiquitination-based indicator, enabling the cell cycle phases to be monitored in real-time.

- HT1080 cells, stably expressing the indicator were treated with either cisplatin or fluorouracil (5FU), imaged every 2 h (10x).

- Objects classified based on red and green fluorescence.

- Cisplatin drives the cells to S/G2/M (green) phenotype, consistent with stalling at DNA replication.

- 5FU stalls DNA synthesis and drives the population to a G1 (red) phenotype.

IncuCyte® System for Continuous Live-cell Analysis: Methodology

IncuCyte® 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.
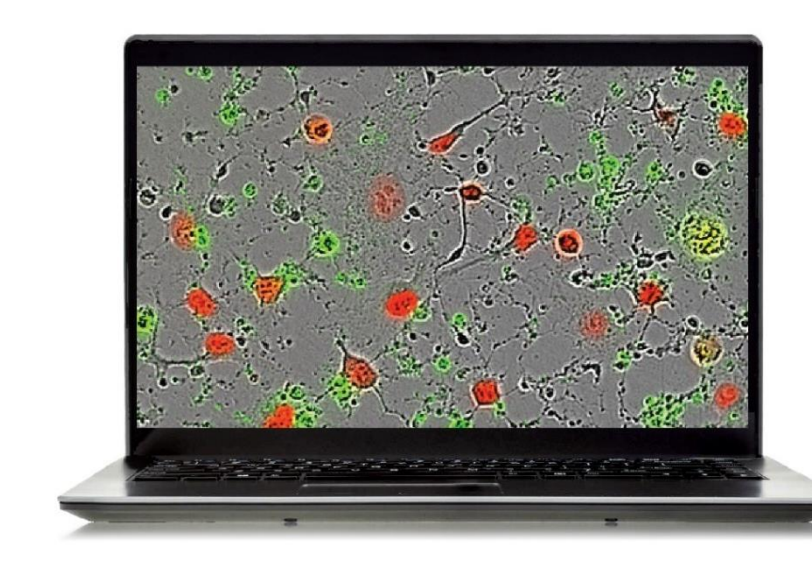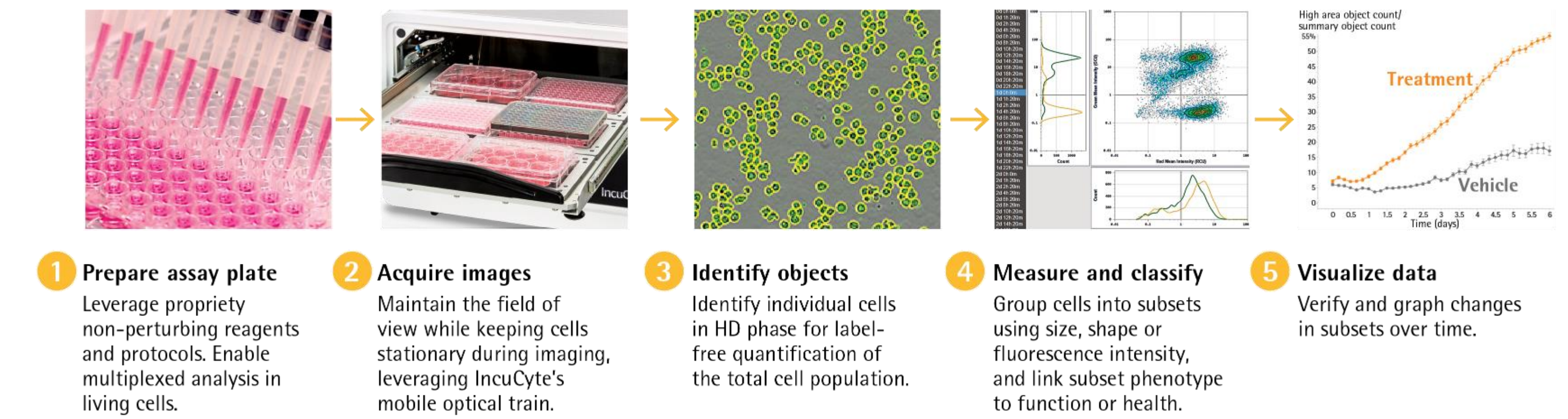
IncuCyte® Software
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.
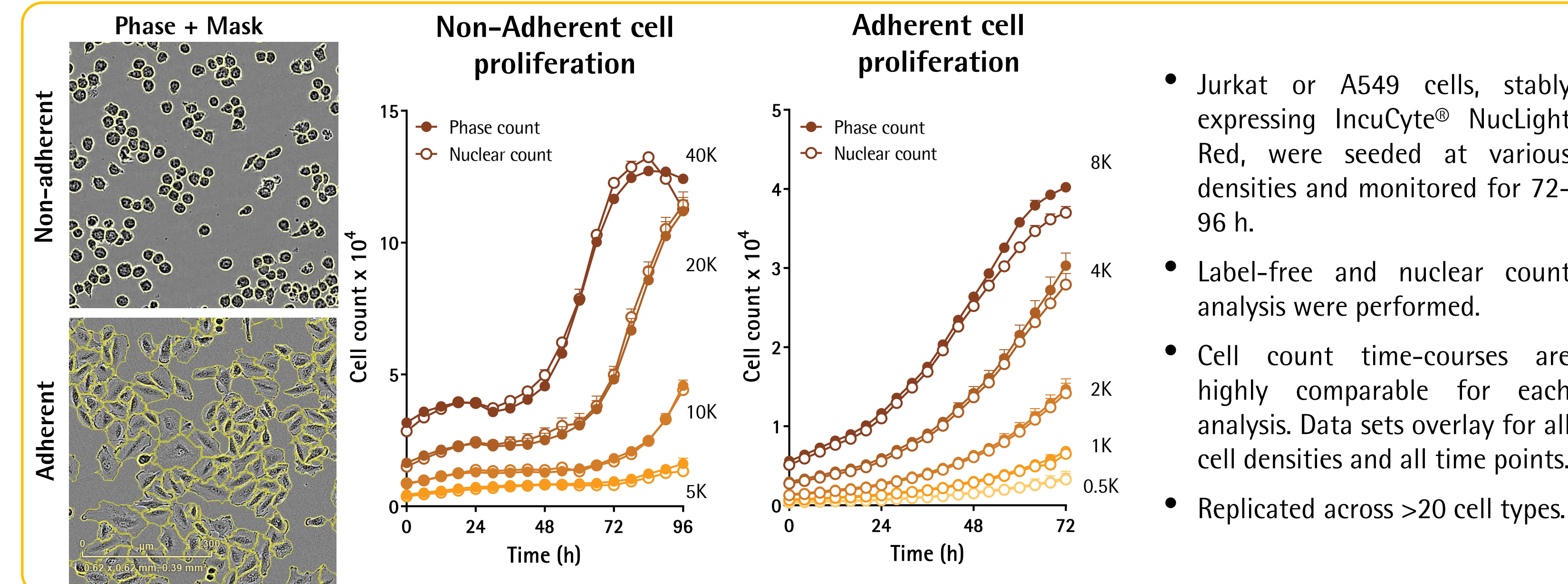NEW: IncuCyte® Cell-by-Cell Analysis Software Module
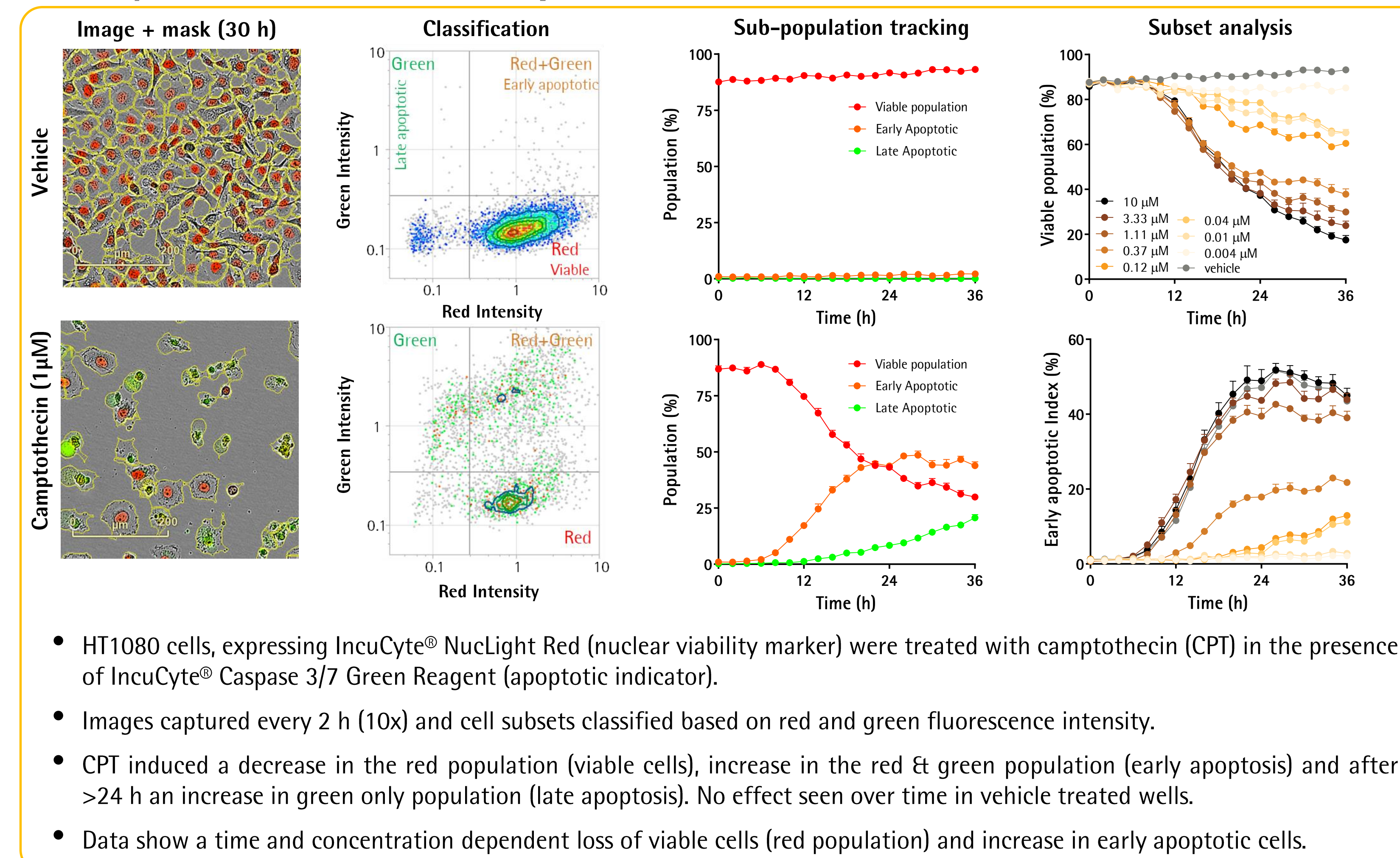
IncuCyte® Reagents and Consumables
A suite of non-perturbing cell labeling and reporter reagents. Includes nuclear-targeted GFP and RFPs for cell counting plus no-wash cell health reagents for apoptosis and cytotoxicity.

1 **Prepare assay plate** Leverage propriety non-perturbing reagents and protocols. Enable multiplexed analysis in living cells.

2 **Acquire images** Maintain the field of view while keeping cells stationary during imaging, leveraging IncuCyte's mobile optical train.

3 **Identify objects** Identify individual cells in HD phase for label-free quantification of the total cell population.

4 **Measure and classify** Group cells into subsets using size, shape or fluorescence intensity, and link subset phenotype to function or health.

5 **Visualize data** Verify and graph changes in subsets over time.

Label-free, accurate cell segmentation

- Jurkat or A549 cells, stably expressing IncuCyte® NucLight Red, were seeded at various densities and monitored for 72-96 h.

- Label-free and nuclear count analysis were performed.

- Cell count time-courses are highly comparable for each analysis. Data sets overlay for all cell densities and all time points.

- Replicated across >20 cell types.

Phenotyping of HER2 expression, to show heterogeneity

- HER2 expression evaluated in A549, SKOV-3 or SkBr3 cells.

- α-HER2 Ab was fluorescently labeled with IncuCyte® FabFluor-488 reagent.

- Quantification of images demonstrates:
  - A549: lack of HER2 expression
  - SKOV-3: heterogeneous expression
  - SkBr3: homogenous, high expression

- Isotype control responses represent the negative population.

Analysis of cell health: Population identification

- HT1080 cells, expressing IncuCyte® NucLight Red (nuclear viability marker) were treated with camptothecin (CPT) in the presence of IncuCyte® Caspase 3/7 Green Reagent (apoptotic indicator).

- Images captured every 2 h (10x) and cell subsets classified based on red and green fluorescence intensity.

- CPT induced a decrease in the red population (viable cells), increase in the red & green population (early apoptosis) and after >24 h an increase in green only population (late apoptosis). No effect seen over time in vehicle treated wells.

- Data show a time and concentration dependent loss of viable cells (red population) and increase in early apoptotic cells.

Monitoring PBMC activation: morphology & protein expression

- PBMCs (30K/well) were activated with α-CD3 Ab (10 ng/ml) and IL2 (10 ng/ml) and labeled 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.

Specific, non-perturbing fluorescent labeling

- Jurkat and Ramos cell mixtures (0-100%) were plated and monitored over a 48 h period.

- α-CD20 Ab was fluorescently labeled 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.