# High Content Analysis of phenotypic assays in cancer drug discovery

Hajo Schiewe[1], João Carvalho[2], Fleur Stevenhagen[2], Servane Lachize[2], Rhea van de Bospoort[2], Melek Atalar[2], Kimberly Lo[2], Lianne van Beek[2], Marta da Silva[2], Niki van der Steenstraten[2], Viola Tabel[2], Jeroen Esselink[2], Jeroen Overman[2], Ian Gowers[3], Roger Clark[3], Shilina Roman[4] and Shushant Jain[2]
[1]Charles River, Wilmington, Massachusetts, USA, [2]Charles River Leiden NL, [3]Saffron Walden UK and [4]Harlow UK

Contact: UK-EarlyDiscoveryClientServices@crl.com

## 1 INTRODUCTION

Charles River Laboratories (CRL) has developed many disease-relevant cellular models to screen small molecules and genetic modifiers for target and phenotypic based drug discovery. Our expertise and portfolio in bespoke cellular model development covers several therapeutic areas including oncology and a wide variety of target classes.
Complex cellular models derived from primary cells, patient derived embryonic stem cells and induced pluripotent stem cells have been adapted for phenotypic screening. Models have been further developed to produce physiologically relevant multicellular structures, such as 3D spheroids and co-cultures. Combining complex biological systems with multiparametric high content analysis provides detailed single cell quantification of cellular and subcellular biology.
Such validated High Content (HC) assays can be applied to both target and phenotypic based discovery platforms to support preclinical drug discovery and improve translation of targets and compounds to the clinic.


## 2 ALGORITHMS DEVELOPED IN ONCOLOGY

| Formats | Assays developed |
|---|---|
| Cell viability and synergistic cytotoxic studies | 2D: Clonogenic assay, cell viability assay (Hoeschst/PI), immunotherapy cytotoxic co-culture assays |
| Anchorage independence and colony forming assays (in 3D) | 3D: Multiple cell lines and co-culture |
| Apoptosis, cell death, DNA damage response, cellular stress and degeneration | Early and late stage apoptosis markers, mitochondrial function, nuclear condensation, DNA damage assay and downstream signaling assays, foci, stress granules, protein inclusions |
| Cell cycle | Cell cycle progression and S phase |
| Cell motility and migration | Scratch wound assay and Boyden chamber motility assay |
| Acquisition of invasive properties | Epithelial to mesenchymal transition (EMT) assay |
| Post translational modification | Multiple assays for signalling events (phosphorylation and acetylation) |
| Marker expression | Multiple assays to assess transduction efficiency, epigenetic target and biomarker expression |
| Receptor internalization and degradation | Nuclear receptor degradation, receptor internalisation, ligand binding |
| Translocation of transcription factors | Multiple cytosolic/nuclear translocation assays |

## 4 INSTRUMENTS/DATA ANALYSIS


IN Cell 2200


IN Cell 6000


IncuCyte S3

Our extensive experience in the development of complex cellular assays:
Execution of high-throughput phenotypic screenings involving multi-parametric readouts, design of quantitative image segmentation algorithms, and experience in a wide variety of disease models (incl. co-cultures, 3D and organoid systems) allows us to approach with confidence a diversity of preclinical drug discovery programs.
Multi-parametric feature extraction is performed by using analytical software tools such as IN Cell Developer Toolbox, IN Cell Analyzer Worstation and Cell Profiler™.

## 3 EXAMPLES OF HIGH-CONTENT BASED ONCOLOGY ASSAYS

### SCRATCH MIGRATION ASSAY

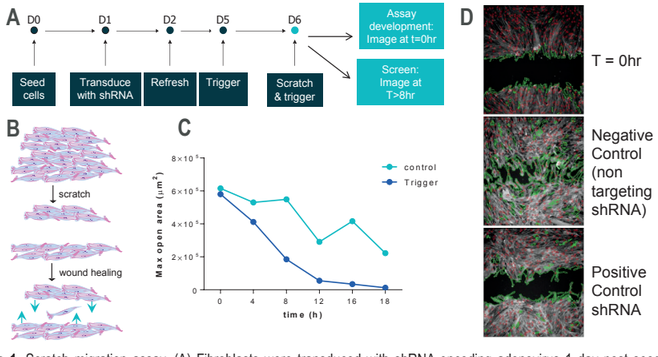



**Figure 1.** Scratch migration assay. (A) Fibroblasts were transduced with shRNA encoding adenovirus 1 day post seeding, after 4 days of treatment, the cells were triggered with PDGF-BB. A scratch was created 1 day post trigger addition. (B) Graphical representation of the migration of fibroblasts into the open area created by the scratch. (C) Trigger induces full wound closure after 18h. (D) Example of images depicting migration into open area and inhibition of cell migration. This assay uses a high content algorithm developed to segment and quantify the open area to identify migration inhibition by target gene knockdown.

### IMMUNO-ONCOLOGY 3D SPHEROID T CELL CYTOTOXIC ASSAY

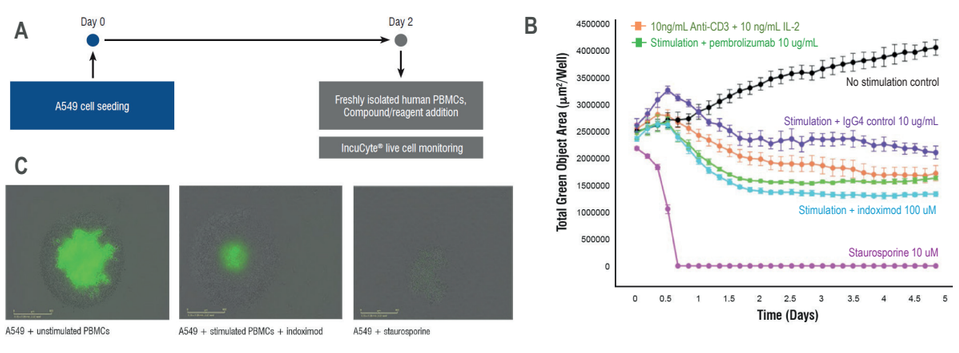
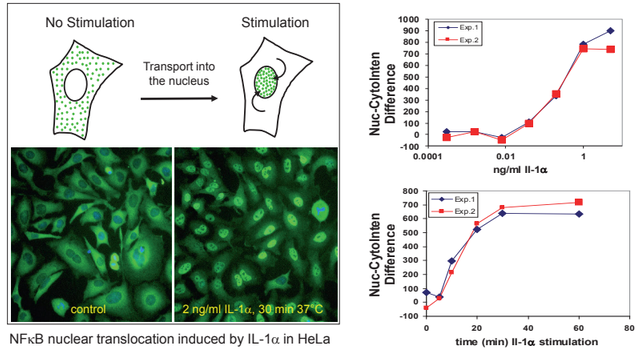



**Figure 2.** 3D Spheroid T cell cytotoxicity Assay. (A) A549 cells were transfected with IncuCyte® Nuclight Green lentivirus reagent to generate a stable cell line. Cells were seeded in ultra-low attachment plates to enable spheroid formation, and treated after 2 days of culture with activated T cells in the absence and presence of test compounds or therapeutic antibodies. This method uses the IncuCyte® live-cell analysis system to generate data in the form of presentation-ready and time-lapse graphs and movies. (B) Example of data generated with IncuCyte® over a period of 5 days after treatment. (C) Examples of live cell imaging acquired with IncuCyte®.

### NUCLEAR TRANSLOCATION ASSAY




NFκB nuclear translocation induced by IL-1α in HeLa

**Figure 3.** Nuclear translocation assay. NF-κB signaling in inflammation and cancer. In the control situation NF-kB is detected in the cytoplasm and not in the nucleus. Once cells are treated with IL-1α, nuclear translocation of NF-kB is evident. Using HC analysis the concentration- and time-dependent effects of IL-1α in the nuclear translocation of NFκB could be studied in HeLa cervical cancer cells by ratiometric quantification of NF- κB expression in the nucleus versus the cytoplasm