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HIGH CONTENT SCREENING AND THE CELLCHIP™ SYSTEM: LIVING CELLS AS BEACONS FOR DRUGS AND TOXINS

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INTRODUCTION: The new face of drug discovery is focused on the living cell with its myriad ionic, metabolic, macromolecular, and organellar networks as the ultimate target of drug activity. Cellomics® has developed 'High Content Screening' (HCS) technology1,2,3 for the automation of information-rich cell based assays using an integrated platform comprised of imaging instrumentation, fluorescent reagents, engineered cells, and bioinformatics tools. HCS is defined as multiplexed functional screening based on fluorescence imaging of multiple targets in the context of intact cells. This novel approach measures temporal and spatial distributions and activities of targets and cellular constituents in and between cells. An HCS multiparameter assay yields 20-200 target-related measurements on up to four different cell targets, each of which is identified on a separate fluorescent channel. Drug or toxin effects on complex molecular events such as signal transduction pathways can be measured, in addition to effects on cell functions ranging from apoptosis to cell-cell communication. The unique advantages of HCS are manifold. High-content screens extract high quality information on a cell-by-cell basis rather than providing an average population response measurement. Furthermore, individual cells of interest are distinguishable within a mixed cell population. HCS eliminates potential sources of variability including those associated with pipetting error, transfection efficiency, and cell preparation. Interactions between drug candidates, or toxins, and multiple cellular targets, as well as downstream events, can be monitored in a single HCS assay via multicolour fluorescence. High-content screens can be performed using fixed or live cell formats to yield the temporal-spatial dynamic information necessary to determine the role of selected targets in cell functions and the specificity of drug compounds or toxins. The evolution of HCS into a higher throughput and miniaturized platform will be realized with the CellChip™ System4 under development.

METHODS: By harnessing the power of fluorescence imaging, bioinformatics, robotics, and advances in cell biology we have developed the High Content Screening (HCS) method. At the core of HCS is the multiparameter image analysis of numerous whole cells in parallel which provides extreme flexibility, speed and capacity to quantify complex biology

The ArrayScan® II, optimised for fixed endpoint assays enables HCS on living cells. The ArrayScan II System's unique optical path is optimised for performing rapid automated scans through the bottom of clear-bottom microplates. The system automatically focuses on a field of cells and acquires images at each selected colour channel. The ArrayScan software identifies and measures individual features and structures within each cell in a field of cells, so that hundreds of cells are analysed in parallel. The software then tabulates and presents the results in user-defined formats. All of the raw data-including images of individual cells-are archived and available for inspection and analysis. The ArrayScan II software provides for multicolour imaging, automated cell-based image analysis, and data management for archiving, analysis and creation of reports. An intuitive, graphical user interface guides you through image acquisition, image analysis, data review and data reporting.

RESULTS: High Content Screening enables a quantitative analysis of multiple intracellular pathways affected by interaction with drugs or toxins. Intracellular cytoplasm to nuclear translocation of transcription factors, receptor internalisation, discrimination of apoptotic cells from necrotic cells, and functional measure of drug or toxin induced cellular toxicity are some of the measured HCS parameters for functional assessment of change in cellular physiology induced by drugs or toxins. High Content Screening, applied to lead optimisation, predictive toxicology, and new target validation can ultimately reduce the "idea to clinic" cycle time while increasing the probability of therapeutic success of leads.

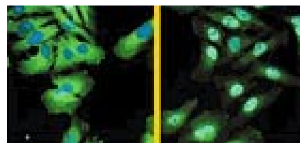

Figure 1. Images of immunofluorescent stained HeLa cells before and after activation of NFκB by IL-1i. Nuclei (blue) are labelled with Hoechst dye. Cells were stimulated with 25 ng/mL IL-1i for 20 min. LEFT: NFκB labelling in unstimulated cells (green). RIGHT: NFκB labelling in stimulated cells (green).

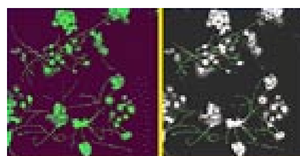

Figure 2. Application of the Neurite Outgrowth imaging algorithm on a field of PC-12 cells acquired with a 10X objective lens on the ArrayScan II System. The left image displays a composite of the nuclear and neuronal raw images acquired by the ArrayScan II System. Cell nuclei (blue) are labelled with Hoechst Dye. The neurons and their neurites (green) are identified by immunofluorescence. The panel on the right shows the neuronal image in greyscale, with the neurites identified by the algorithm overlaid with a green trace.

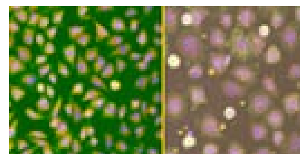

Figure 3. L929 cells treated with 2.5 μM paclitaxel for 30 hours (right), and untreated (left). F-actin (Ax488-ph) appears in green, nuclear staining (Hoechst) appears in blue, and mitochondria (MitoTracker® Red) appear in red. Images taken from the ArrayScan II System.

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