5D VIRTUAL MICROSCOPY AS AN ALTERNATIVE TO HCA IN HIGH CONTENT SCREENING FOR NANOPRODUCT AND ENVIRONMENTAL WASTE TOXICITY

Kris Ver Donck1, Marc Moeremans1, Luc Bols1, Yves Willems1, Reinhalde Weltens2, Hera Lichtenbeld3 & Johan Geysen1


1. SYNOPSIS

In the drug discovery R&D workflow, high content analysis (HCA) is in essence a linear, repetitive process. Compound libraries are screened for affecting one (or at most a few) rigorously validated cellular features in a cell-based assay (Panel 2, top). In this linear workflow, image analysis reduces image information into numerical componentality and the process is ruled by robust reproducible performance (high Z) and leaves little, if any, room for serendipitous observation. Also biomanufacturing, cell therapy and stem cell R&D workflows in essence are linear (Panel 2, middle).

In high content screening for toxicity of e.g. nanoproducts and complex environmental wastes, cytotoxicity materializes to achieve HCA speed while maintaining serendipity, the high content microscopy reader workflow was expanded to provide full multidimensional image data sets from all wells of multiwell plates (XYZ, spectral modes, time). As all image data are ported to the PC, the scientist can review and use at a high speed efficient and in a much shorter period of time, compatible with plate cycle image capture speed (Panel 3). All spectral microscopy modes (multiple fluorescent and brightfield combined) can be evaluated simultaneously in overlay views. The scientist can focus by scrolling Z-stack images. All multimode time lapse sequences taken in parallel can be reviewed as by review events across the time dimension. Calculations have shown that the speed of observation using virtual microscopy software is up to 20-fold faster or less than 5% of the time required at the microscope with the samples and even much faster in time lapse experiments. Examples demonstrate how reference and experimental nanomaterials of defined sizes display time and size-dependent toxicity as well as specific cellular response over time (Panel 4); how the physicochemical behaviour of environmental waste samples induces local early-onset cytotoxicity (Panel 5) and in cell proliferation assays, how label-free, live cell counting with image analysis and mitochondrial assays are ranking waste water samples identically (Panel 6).

4. NANOPRODUCT CYTOTOXICITY

Summary level data of a comparison of an experimental 1.4nm metal colloid nanoparticle with a differentially sized reference colloid metal nanoparticle. From top to bottom, these time point additions were shown for a high dose after 3hr and a 10-fold lower dose 24hr & 96hr. From left to the right by caption: “Control”: solvent controls; “Ref. Comp.”: an IgG (rabbit) reference control with known cytotoxicity effects on normal human keratinocytes; “1.4nm”: treatment with 1.4nm colloid metal particle, coated with ligand; “1.4nm-IgG”: the ligand used to coat the particle at a 10-fold overdose versus the particle; “10hmM-NaCl”: reference metal colloid particle (same metal) with a 10-fold higher diameter than the experimental 1.4nm particle. In 98%, solvent treated normal human keratinocytes grow from 10% confluent to 100% confluent, whereas cells treated with reference compound grow to 50% confluence and the cells display hypercalcification, they are bigger or flatter than control cells. The 1.4nm colloid metal particle, coated with ligand kills the cells in less than 3hr at high dose and within 24hr at 10-fold lower dose, whereas the IgG solution does not affect cell growth or morphology in a 10-fold higher dose. When encapsulated and colloidal nanoparticles, cells survive, but not proficiently display a phagocytic behaviour as phagocytosis they clean up the particles in the direct environment as long as they carry the particle load.

Different high content workflows. Linear workflows: the materials (left green boxes) lead to a HCS assay or transduction step, followed by a “many to one” process such as high speed screening, cell line or colony selection (orange box), after which the selected items are studies extensively in a “one to many” process. It is in “one to many” repetitive processes that image analysis proves most valuable. Complex workflows: High complexity models (such as in systems biology programs) or cases where unexpected effects occur (toxicity or sample physicochemical behaviour) do not always allow for image analysis. In these cases (yellow area) virtual microscopy creates speed w/o need for image analysis.

5. WASTE WATER RESIDUE TOXICITY – EARLY ONSET

Sample #4 displayed immediate reduction of cell motility in the monolayer, arrest of mitosis and in the vicinity of precipitated sample components, focal cell blebbing and focal cell death. There is moderate migration in the monolayer into the direction of foci of cell death. Methods: Nanocentric human epidermal keratinocyte monolayer (24 Well plate) were exposed to a sample dose titration and transferred to the MAAS-2 reader for Z-stacks and centre well plate time lapse image capture (20-minute cycle). After 24h, images were viewed using the GUI shown in panel 3. Time lapse sequences immediately revealed the precipitating nature of some waste water residue extracts (not shown).

Conclusion

“5D Virtual Microscopy” provides a fast alternative to image analysis in conditions where complex samples (waste water & nano products) elicit complex forms of behaviour in their interplay with cells in cell-based assays.

6. WASTE WATER RESIDUE TOXICITY – IMAGING & MTT

Comparison of mitochondrial assays and image analysis results in keratinocyte growth assays. 5 samples (A, B, G, I, K, A) display similar IC50 values in image analysis and mitochondrial assays. Methods: 2000 human epidermal keratinocytes were seeded in 24-well plates and allowed to adhere overnight prior to addition of the samples or 0.1% DMSO. Images were captured for 5 consecutive days and then the cells were sacrificed for an MTT assay.

For full video demonstration please refer to authors at booth #273.