

Rapid Non-Invasive Assessment of Compound-induced effects on Growth of Unlabelled Primary Human Cell Cultures

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A variety of surrogate endpoint assays (e.g. fluorescent or mitochondrial assays) have been developed for screening compounds or genes with modulating effects on cell proliferation. All these assays are either destructive or interfere with the biology of the culture. From a process point of view, the addition of assay reagents, incubation steps and plate washings is of high complexity and cost intensive.

A current trend in drug discovery and development is to include multiple endpoints in cell-based assays. It has created a need for simpler, non-invasive and faster assays for assessing the cell number in multi-well plates.

We have developed automated brightfield imaging methods to detect living unlabelled cells using the MIAS[®]-2 microscopy reader and newly developed eaZYX[®] imaging application software. Two essential features of the application are: (i) fast object-based auto-focusing allowing to capture high quality images and (ii) the use of cell detector principles for adherent cells that are independent from air-liquid meniscus induced variable backgrounds and therefore applicable to whole well scanning.

In this poster, a fast non-invasive confluence application was used to study the effects of drug candidates on cell growth in primary cultures of living human epidermal keratinocytes. The IC₅₀'s derived through imaging and mitochondrial assays matched very well. The non-invasive character of the application further allowed multiple serial observations on the same batch of plates, facilitating the assessment of compound effects over time. The image-based documentation tools (e.g. plate mosaic overviews) allowed identifying toxic compounds from non-toxic ones in the same experiment.

Single image capturing took about 3 seconds per well resulting in a plate cycle time of less than 5 minutes for a 96 well plate. Object identification and confluence assessment was executed in 45 seconds. For whole well scanning of 16 images/well (4x objective) and 25 images per well (5x objective) the plate cycle times were 12 and 20 minutes respectively. In contrast, mitochondrial assays require a total assay time of >3 hrs. Similar applications have been developed for robust confluence detection of the adherent cell lines CHO, HEK-293, 3T3, HeLa.

The non-invasive character of the methods allows for a broader applicability in combination with existing functional cell-based assays and screens.