

RAPID NON-INVASIVE ASSAYS FOR CELL GROWTH AND INHIBITORY EFFECTS OF COMPOUNDS ON PRIMARY CULTURES OF HUMAN UNLABELED CELLS

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The interest in automated cell based assays has greatly increased because of the development of high information content screens using fluorescent techniques. The availability of molecular biology tools to mark in situ all kind of proteins with a fluorescent tag has allowed studying their role in complex biological phenomena, such as signal transduction pathways. However there is still a substantial need for generic assays to read additional endpoints in the drug development chain. Preferentially these assays are developed as such that they can be directly integrated into the existing functional screening protocols.

We have developed automated brightfield imaging methods to detect and count living unlabelled cells using the MIAS[®]-2 microscopy reader and newly developed eaZYX[®] imaging routines. Two essential features of the method are: i) the use of autofocusing algorithms based on scale space mathematics allowing to capture high quality images in a fast way; ii) the use of algorithms allowing to analyze images independent of their variable background. The methods proved to be useful to count cells in suspension cultures and to determine the stage of confluence in cultures of adherent cells. Image capturing took about 3 seconds per sample resulting in a plate cycle time of less than 5 minutes for a 96 well plate. This rapid non-invasive cell count and/or confluence application was used to study the effects of drug candidates on cell growth and cell death in primary cultures of living human epidermal keratinocytes.

The generic nature and non-invasive character of the assay allows for intra-assay assessment of toxic effects, as well as of stimulatory/inhibitory effects of compounds on cell growth in a wide variety of existing functional cell based screens.