# Inhibition of Cell Growth by Compounds

## Automated Assessment of Compound-induced Growth Inhibition of Unlabelled Human Epidermal Keratinocytes Using the MIAS®-2 truVIEW Microscopy Reader and eaZYX® Imaging Software

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## Objective

An automated method for the assessment of effects of compounds on cell growth is described. The method requires no label and uses the MIAS-2 truVIEW microscopic reader equipped with eaZYX software. Capturing only center images of the wells in a 96 well plate, at a 5x magnification in brightfield mode, proved to be sufficient to assess the stage of confluence of the cell cultures in an accurate way. After fine tuning, the novel imaging method was used to study inhibitory effects of compounds on the growth of normal human keratinocytes.

## Introduction

Because mitochondrial assays, such as MTT (1), are easy amenable to high throughput , they are often used as surrogate endpoint to determine the growth inhibitory effects of compounds. However it must be emphasized that these methods require several manipulations: a) adding the substrate; b) incubating the cells with the substrate; c) removing the medium; d) dissolving the precipitated formazan crystals before spectophotometric measurement. In this regard, direct and automated image analysis of unlabelled cell cultures could be an interesting alternative.

## Materials/Methods

Normal human keratinocytes, isolated from foreskin, were cultured in keratinocytes serum-free medium (K-SFM, Invitrogen, Cat.No.17005-075) supplemented with hEGF and BPE, under standard conditions (95% RH, 37 ºC, 7.5% CO2). Cells were passaged using 0.025% trypsin in 0.01% EDTA and used in passage 4. The cells were seeded at 2000 cells/well in 200 µl into a 96 well plate (Greiner, Cellstar, Cat.No. 655180). After overnight incubation, compounds were added to the wells in a concentration range. For MTT, duplicate plates were prepared and all plates were incubated for 7 days, until the solvent (0.1% DMSO) control reached 100% confluence. After fixation with 2.5% glutaraldehyde for 15 min at rt, one image (512 x 512 pixels) per well was captured using a 5x objective and 1.0 Optovar settings. A specific confluence application for adherent cells was selected for image analysis. Center-well images with the analysis result overlays were created using eaZYX imaging software (2). Data tables were imported in a

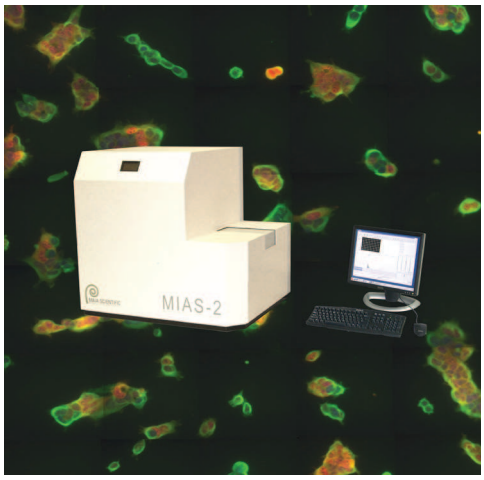


Figure 1. The MIAS-2 reader equipped with eaZYX IMAGING software.

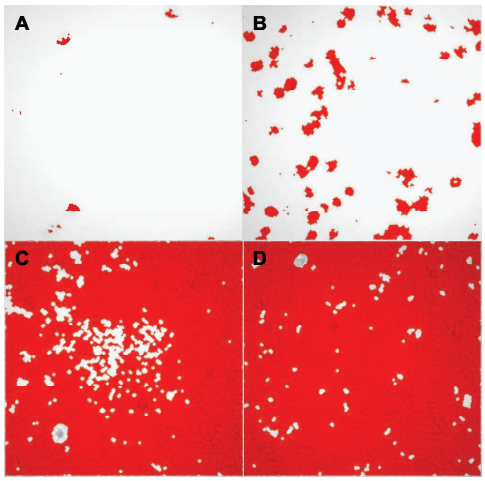


Figure 2. A single image per well showing the cells and the confluence image analysis result (overlay in transparent red). The inhibitory effect of compound 2 is shown for 10µM (A), 3µM (B) and 1 µM (C). Image D is a solvent control (0.1% DMSO).

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spreadsheet program (MS-Excel) and graphics were prepared. The images shown in figures were JPEG compressed and contrast adjusted prior publication. compressed and contrast adjusted prior to publication.

## Results

At a 5x magnification, the plate cycle time for a 96 well plate was ~5 minutes when a single image in the center of the well was captured indicating that imaging took only 3 seconds per well. On these images, the total area covered by the cells was automatically detected and calculated with a specific confluence application. As shown in Figure 2, the resulting binary images matched the area covered by the cells, in sub-confluent as well as in confluent monolayers. Figure 3 (A en B) shows the effect of different compounds on the growth of keratinocytes as obtained with bio-imaging and MTT. No major differences can be observed and this is confirmed in Figure 4 showing the correlation coefficient of the IC50s, as calculated by linear interpolation for both methods.

## Discussion

The results show that the above bio-imaging application can assess in accurate way the compound induced growth inhibition of unlabelled human keratinocytes. The method is fully automated, for image capturing and as well as for the subsequent image analysis using eaZYX software. A major advantage of the assay, as compared to surrogate endpoint assays, is the reduction in hands-on time and consumables. The reduction can be even larger if the fixation with glutaraldehyde is omitted. The latter is only necessary upon handling a large number of plates allowing to synchronize the incubation time with the compounds. In practice, capturing and analysis of only one 512 x 512 pixel image per well proved to be sufficient to obtain accurate data. This resulted in short plate cycle times, a prerequisite for its use in high throughput screenings.

## References


1. Mosmann, T. et all, 1983, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, JIM, 65 (1-2):55-63.

2. Van Osta, P., Geusebroek, J.M., Ver Donck, K., Bols, L., Geysen, J., ter Haar Romeny B. M., 2002, The principles of scale space applied to structure and colour in light microscopy, Proceedings of the Royal Microscopical Society, 37 (3): 161-166.


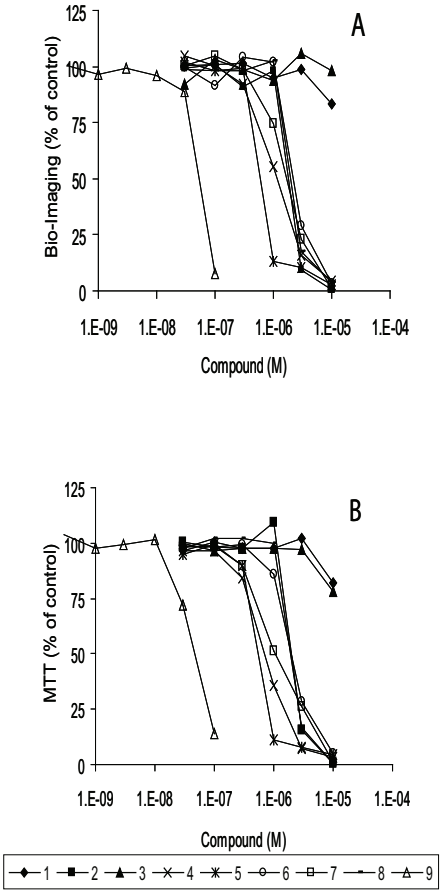


Figure 3. Graphical presentation of the inhibitory effects of different compounds obtained with bio-imaging and eaZYX software(A) and MTT (B). Data are expressed as percentage of the DMSO control.

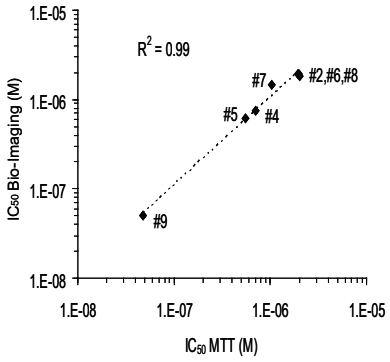


Figure 4shows a perfect correlation between the IC50s obtained with bio-imaging and MTT assay.


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