

Use of a novel, self-learning bio-imaging software to study small molecule induced lipid accumulation in primary cultures of human epidermal keratinocytes

Marc Moeremans, Bieke Govaerts, Luc Bols, Kris Ver Donck, Yves Willems and Johan Geysen

MAIA SCIENTIFIC, Cipalstraat 3, B-2440 Belgium
www.maia-scientific.com and info@maia-scientific.com



The keratinocyte is the main cell constituent of the epidermis. It undergoes a complex and carefully regulated program of proliferation and differentiation throughout its vertical migration from the basal layer to the upper layer of the epidermis. Once arrived in the stratum corneum, the terminally differentiated anucleated corneocytes are first cemented to each other by intercellular lipids to create the skin's barrier function, but ultimately detach or desquamate.

The exact mechanism of the lipid homeostasis during the differentiation process is not fully understood. In several studies the involvement of the nuclear hormone receptor PPAR (Peroxisome Proliferator-Activated Receptor) has been described. Upon studying the effect of small molecule drug candidates on keratinocyte differentiation markers we observed, at the light microscopical level, that some compounds also induced the accumulation of large-size lipid droplets, an observation that was confirmed by Oil Red O histochemistry. Because of the obvious interest in compounds with modifying effects on the barrier function of the skin and the involvement of lipids in this process, a project was started to study these phenomena more in detail.

A primary keratinocyte cell-based high content screening model was developed for large-size lipid droplet formation. From several options such as lipid staining by Oil Red O or fluorescent labeling by Nile Red; identification of lipid droplets in label-free, live cells was the preferred option for development, because of lower assay reagent cost, minimal hands-on time and higher overall speed.

The MIAS[®]-2 microscopy reader was used for automated capture of high-resolution (20x) bright-field images of the cells and droplets. A new, self-learning software was used to develop a high-quality, tailor-made image analysis application without the necessity to be able to acquire complex mathematical skills or the need to build an application macro based on a complex set of object detectors.

Sample images from keratinocyte cultures with and without large-size lipid droplets were used to train the software in recognizing large size lipid droplets. The first training result was visually inspected and refined in successive rounds of training to eliminate unwanted false positive detections in a stepwise fashion. Application development was completed in a few hours. The new application was saved and applied to images from compound dose titration and treatment time series of compound-induced lipid droplet formation. The application was capable of discriminating large-size lipid droplets from membrane blebs that were induced by some of the compounds.

This new self-learning software is a very user-friendly new tool for the biologist to build new image analysis applications without the need to acquire the necessary development skills to work with high end bio-imaging software.