Brightfield Image Analysis as a Label-free Adjunct to Fluorescent High Content Screening Assays for Improved Assay Performance & Data Quality

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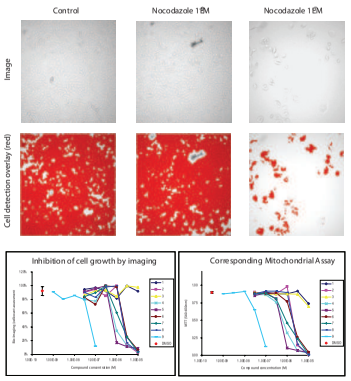
Abstract

High Content Screening has mostly been focused on fluorescent applications.

Brightfield image capture and analysis in combination with fluorescence applications, can provide a better assessment of cell function while reducing the number of (successive) staining procedures.

Unlabelled live cell analysis, repeatable assays on the same cell cultures, clonal assessment and cell count related to these new developments are reviewed here.

Compound-induced Inhibition of Cell Growth by Confluence Image Analysis on Label-free, Live Cells



Legend Brightfield images (top) and transparent overlay with image analysis result in red (middle) for solvent treated (left) and nocodazole treated (middle and right) keratinocytes. At the bottom, inhibition curves are shown for experimental compounds (1 – 8), nocodazole (9) and solvent (DMSO).

Methods:

Primary human epidermal keratinocytes were seeded at 2000 cells/well in 96-well plates and incubated at 37°C and 7% CO_2 in KFSM.

After 24h incubation, the test compounds were added at the final concentrations indicated in the graphs.

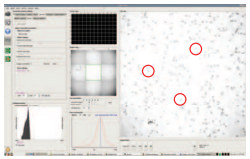
At days 1, 2, 3 and 4 of incubation, brightfield images were captured from the the label-free, live cells with a 5x objective with the MIAS-2® reader.

Images from day 4 were analyzed for culture confluence using the eaZYX Cellcount image analysis application.

The image analysis result was visually audited using red overlays on the images (see images above).

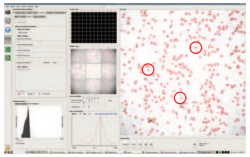
The plates were then processed with MTT to assay for total mitochondrial activity. The formazan signal was read on a ThermoMax colorimeter (Molecular Devices).

Calcein Spiking of Suspension Cells Total Cell Count WITHOUTNuclear Staining

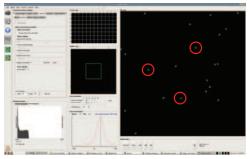


(1) Nine brightfield images of U937 cells in the centre of a well of a 96 well plate shows all the cells in the culture.

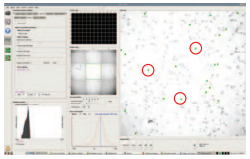
The red circles indicate for reference three cells that are labelled with calcein.



(2) The cell count image analysis application identifies >99% of the cells present in the culture and projects the image analysis result on the image as a red transparent overlay.



(3) The corresponding nine images taken in the green channel show the image analysis result for calcein labelled U937 cells.



(4) When the calcein cell detection image analysis result overlays on brightfield image, the number of "spiked" cells in a culture can clearly be visually assessed.

Methods:

U937 cells were grown in complete RPMI medium. Cells in log phase were centrifuged at 150g_{max} for 5 minutes at room temperature. The cell pellet was resuspended in HBSS supplemented with 1 mM sodium pyruvate to a cell density of 100,000 cells per ml and split in two fractions.

After 30 min, one fraction of the cells was labelled with calcein at a final concentration of 10 µM for 30 minutes at 37°C while the other fraction was left untreated.

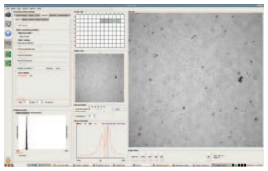
After incubation, both calcein-labelled and unlabelled fraction were again centrifuged and resuspended as above.

The non-labelled fraction was dispensed and spiked with a two-fold dilution series of the calcein labelled cells the calcein-cell-spiked mixtures were seeded in 96 well microClear plate (Greiner).

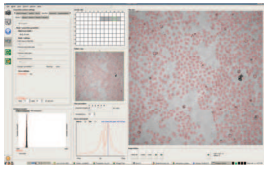
After settling of the cells, images were captured with the MIAS-2® reader using a at 10x objective in two modes, a FLU modes for detection of calcein and a 1 BFD mode for the detection of all cells.

BFD and FLU images were analysed and overlay images generated using eaZYX® software.

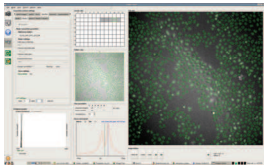
GFP Measurement on Live Unstained Adherent Cells WITHOUT Nuclear Staining



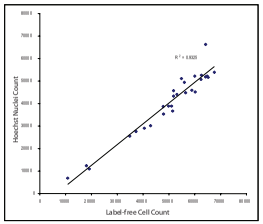
(1) The brightfield image of adherent HELA cells displays the culture as seeded in a 96 well plate.



(2) A transparent red overlay of the image analysis result of the cell count application indicates the cell count result in the brightfield image.



(3) By viewing the B/W fluorescent image under a green overlay for cell count, the number of fluorescent cells can be visually assessed.



(4) Graphical display of the cell count from the label-free, brightfield image (X-axis) versus the Hoechst dye nuclear count (Y-axis).

Methods:

A proprietary GFP::XXX transfected HeLa cell line was grown in DMEM supplemented with 10% FCS, penicillin/streptomycin and 0.3 mg/ml G418.

Cells were seeded in 96 well microClear plates (Greiner) and incubated for 24 hrs at 37°C, 5% CO_2 and 95% relative humidity.

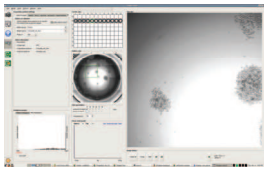
Images were captured at with the MIAS-2® reader using the 10x objective in green fluorescence mode for the GFP signal and in brightfield for detection of all cells.

BFD and FLU images were analyzed and overlay images generated using eaZYX® software.

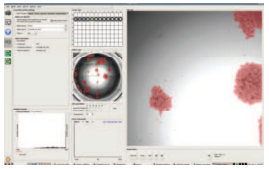
For correlation with nuclear count, the cells were fixed and the nuclei stained with Hoechst dye (Invitrogen, Cat.Nr. 33342) at a final concentration of 5 µg/ml for 60 min at 37°C.

Images were captured with MIAS-2 and analyzed using eaZYX® software.

Label-free, Live Cell Colony Size and Count



(1) Brightfield images of a 12 full-well scans showing a well with several colonies suspension cell seeded in a 96 well plate.



(2) In red overlay on the brightfield images is the image analysis result showing the detected colonies.

Methods:

Suspension cells were grown in serum free medium. Cells were seeded in a dilution series down to 0.5 cells/well in 96 well CellStar plates (Greiner).

Incubation lasted for 3 weeks at 37°C, 5% CO_2 and 95% relative humidity.

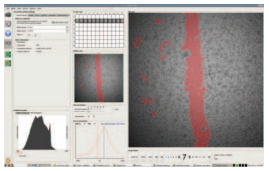
At regular intervals during the clonal growth of cultures, full-well brightfield images were captured from the label-free, live cells with the MIAS-2® reader using the 4x objective.

BFD images were analysed for colonies and overlay images generated using eaZYX® software.

For each time point of image capture and analysis, number, size and position of each colony was reported at each time point.

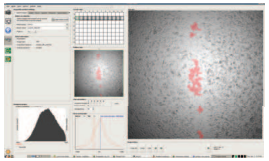
Clonality for each well was determined based on the number of growing colonies in each well.

In Vitro Label-free, Live Cell "Scratch Assay" for Cell Migration Studies



(1) First brightfield image of confluent monolayer grown for 24h in a 96 well plate, immediately after producing the scratch mark.

In transparent red the 'fresh scratch mark' is detected.



(2) Second brightfield image of a confluent monolayer grown for 48h in a 96 well plate and 24 h after making the scratch mark.

In transparent red the remainder of the 'scratch' mark is detected.

Methods:

Adherent cells were grown medium with 10 % serum.

Cells were seeded at high density to form a confluent monolayer in 96 well CellStar plates (Greiner) and were incubated for 24h at 37°C, 5% CO_2 and 95% relative humidity.

A scratch mark was automaticaly generated at the centre of each well using a Plate Scratching device.

Brightfield images were captured on label-free, live cells with the MIAS-2® reader using the 5x objective at two time points: (1) immediately upon generation of a scratch mark and (2) 24h after the generation of the scratch mark.

BFD images were analysed for the size of the scratch mark using eaZYX® software.

Regeneration of the scratched monolayer was calculated from the decrease in size of the scratch mark over the 24h of incubation.

Conclusion

- Cell count & confluence image analysis on label-free cells has been used to replace typical fluorescent or colorimetric assays for assessing cells in wells
- Because they are label-free alternatives applicable to live cells, they allow multiple assessments of the same culture over time and as such minimize the need for plates
- Because they work adequately and in a background-insensitive way, they work well with full well image capture and as such provides representative numbers for the entire well surface
- In combination with fluorescent cell-based assays, brightfield imaging simplifies assay work flow
- Applicability includes (for a variety of both adherent and suspension cell types):
  Cell counting | CIture confluence | Cell viability | Cell growth | Inhibition of cell proliferation | Compound cytotoxicity | Monolayer regeneration | Clonality | Colony size | …
- Brightfield, whole well image capture and analysis is as accurate as e.g. nuclear counter staining or mitochondrial assays and avoids the additional step of staining the cells with a nuclear dye.

Presented at: SBS 2008, St Louis, April 2 10th