Application Note

Growth Rate Monitoring in Cell Line Development

Introduction

The dynamic, label-free monitoring of growth rates in cell line development is important for a number of different workflows, including the following:

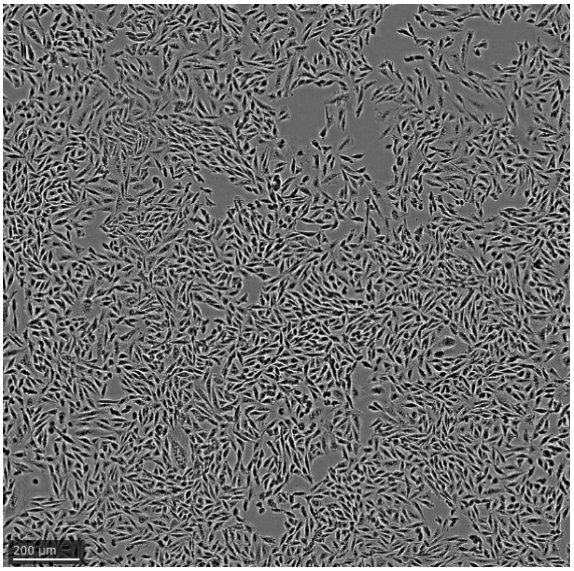
- To monitor successful colony outgrowth from single cell seeding
- To monitor confluence as an indicator for cell growth rates in liquid media
- To identify wells for colony formation from transfections under selective drug pressure e.g. virus vector production

Materials and Methods

The Cell Metric™ imaging platform was used throughout these applications. This is a bright field imager that delivers rapid whole-well images for all plate formats (from six to 384-well plates) and small flasks used in cell line development. Imaging was conducted without labels and without removal of any samples. Cells are typically imaged in flat bottom wells, which encourages cell growth, and the Cell Metric is capable of imaging either adherent or suspension cells. Plate read time is rapid at approximately three minutes in 'growth rate mode' and the output metric selected can be configured to report either confluence (%) or colonies per well depending on workflow and the software application. Plates are barcoded and growth curves for the individual wells are automatically updated each time a plate is analysed.

Figure 1: A) Bright field image of highly confluent cells (note the high definition image quality and the even illumination); B) Software overlay to measure confluence (monolayers are shown in false colour green; over-confluent regions are shown in red).

A)



B)

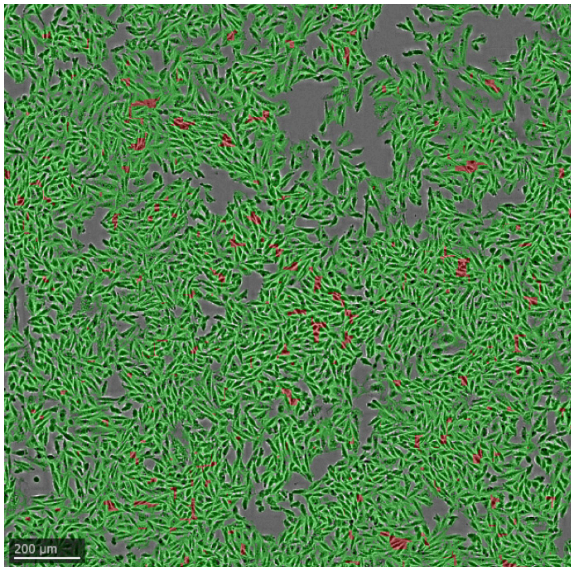


Figure 2: Imaging of CHO K-1 cells.

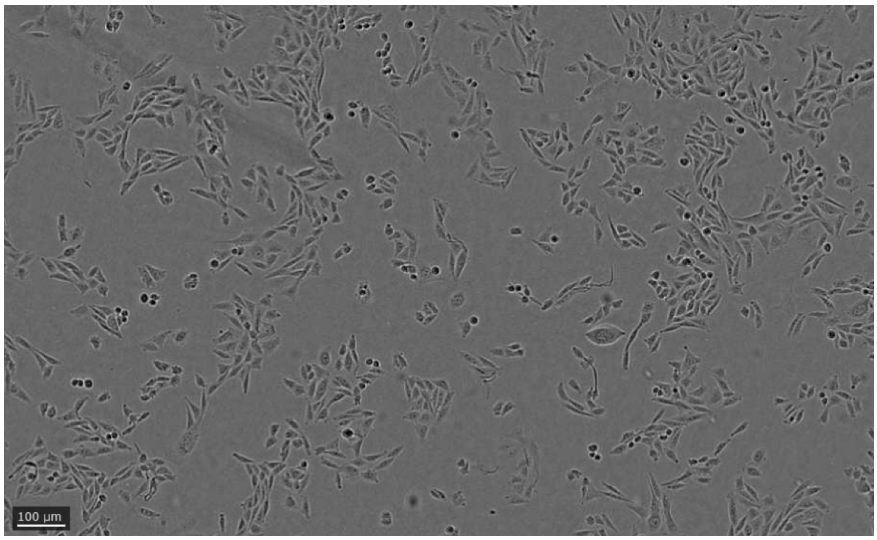


Figure 3: Imaging of HEK293 cells.

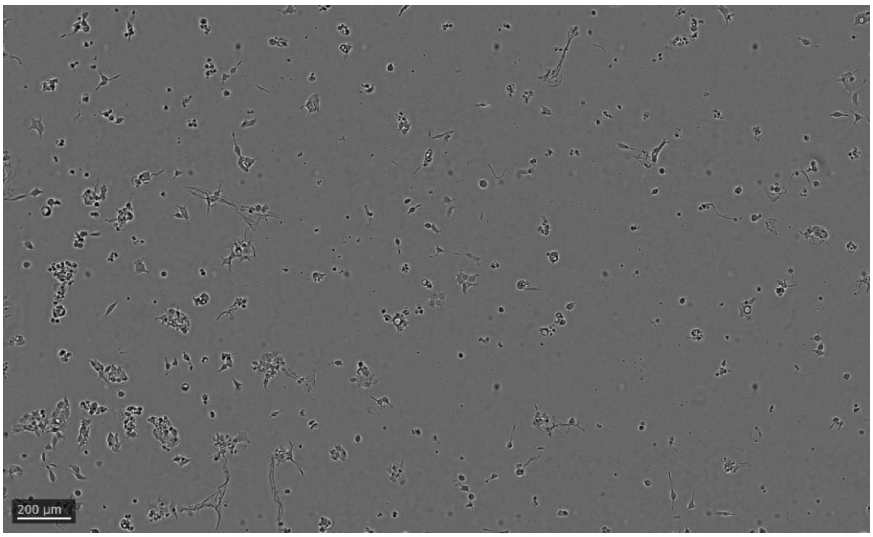
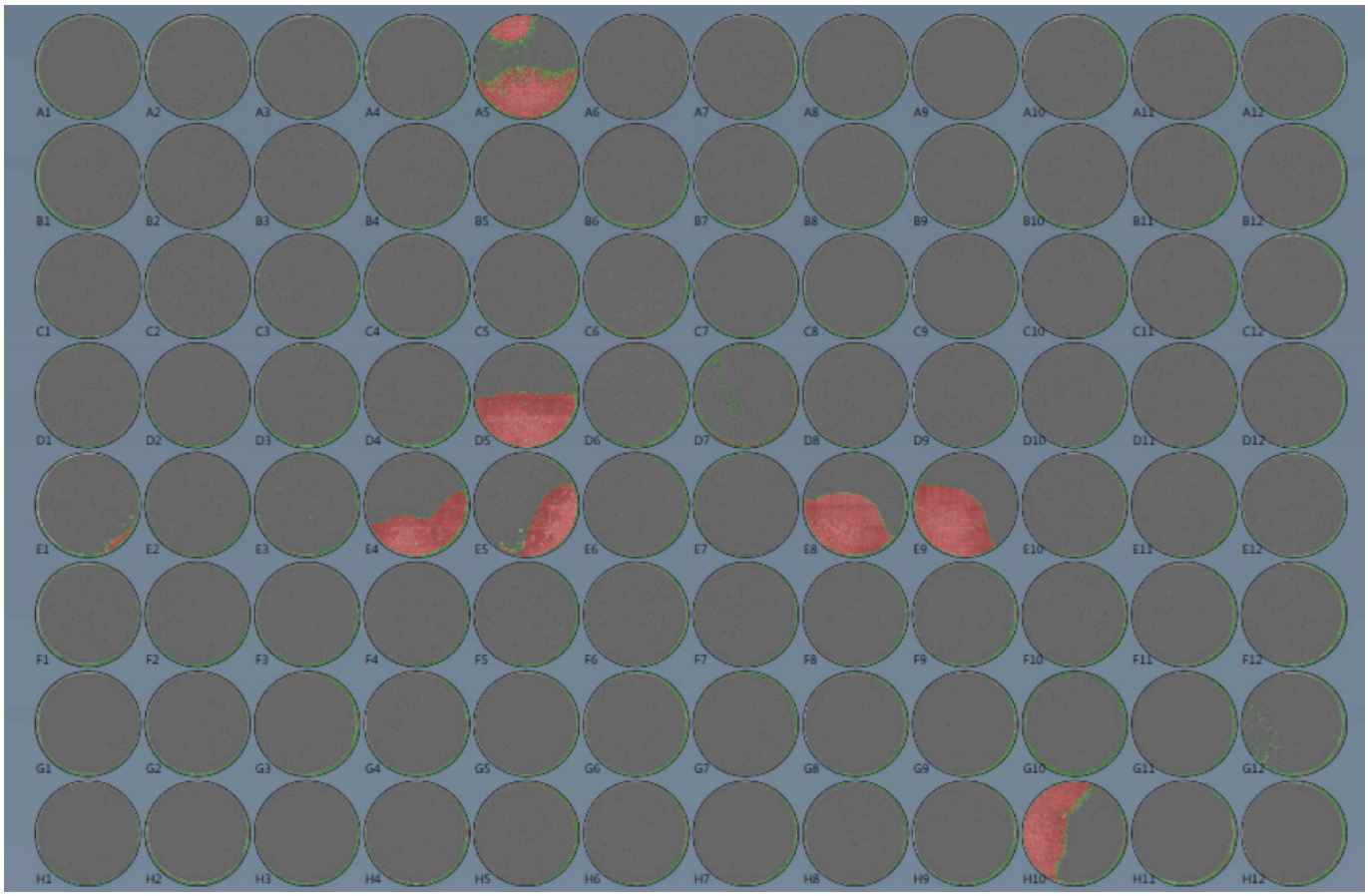


Figure 4: 96-well plate overview for colony outgrowths detected using confluence (day 14 after seeding). A false red colour overlay specifically highlights the seven wells in which colonies have formed.



Data and Discussion

General imaging for confluence

Whole-well imaging of cells can be conducted under bright field conditions with subsequent false colour overlay for enhanced analysis (**Figures 1–3**).

Imaging confluence for colony outgrowths from single cell seeding

Imaging takes place in the cell line development workflow, allowing the scientist to analyse and decide which wells have formed outgrowths. Then, coupling this with productivity data, they will review the single-cell images on the day of seeding (see Application Note 1: Clone Imaging and Proof of Monoclonality) for this subset of wells.

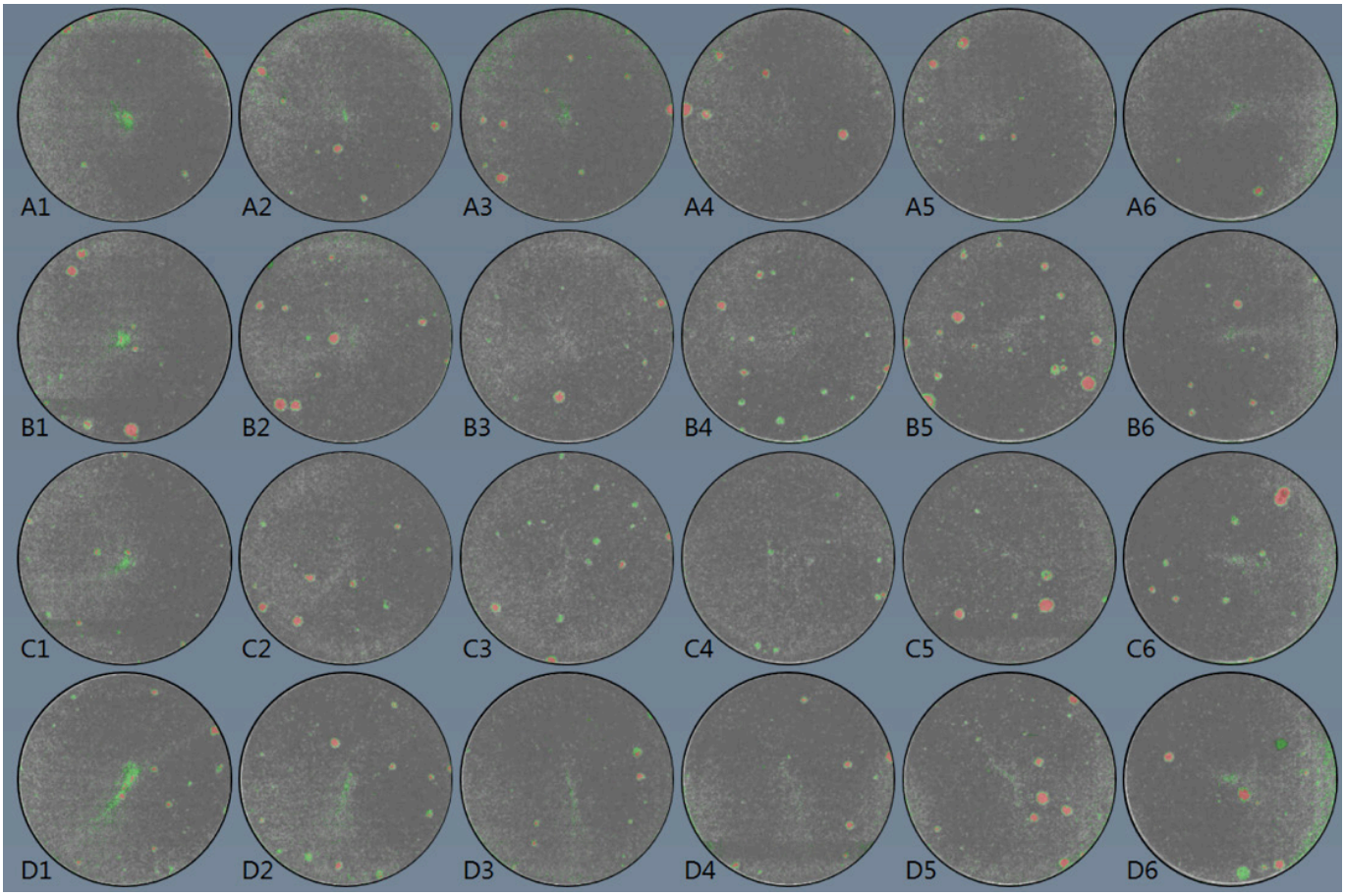
In **Figure 4**, the plate map clearly shows wells in which outgrowths have formed by day 14–21; a table is also generated which provides the user with the percentage confluence and growth rate curves for each well (data not shown).

Imaging for colony identification from transfections

In this application, drug selection is applied to transfected cells that have been plated out. Users are looking for those wells in which colonies form, so that they can subsequently be taken forward for productivity screening and sub-cloning. The challenge in this application is accurate colony detection on a carpet of dead cells, which requires the use of the proprietary high contrast imaging mode on the Cell Metric.

It is worth noting that this application is also highly relevant to vaccine development groups, enabling the effective generation and selection of viral vector producers.

Figure 5: False colour overlay thumbnail images of a 24-well plate showing cell growth/colony formation under selective growth medium.



Conclusions

The Cell Metric imager has been specifically developed as an analytical tool for cell line development, providing a rapid, user-independent and objective measurement of cell growth characteristics in liquid media. These data can then be used as part of the decision-making process when choosing which wells to progress further.

The Cell Metric can bet set up and optimised for different user profiles in the software based on different cell types, morphology and plate types. These profiles are stored by the user allowing for quick access at a later date when these cell types are specifically used.

The decision making process can range from a binary decision (i.e. whether a colony formed or not), to a relative growth rate assessment based on a percentage confluence threshold value achieved over several days/weeks. In cell line development, only those cell lines which show good growth metrics combined with high titre of the target product will be progressed.

Data management tools on the Cell Metric allow wells to be easily selected for further processing or 'hit' selection based on specific criteria, for example, based on those wells achieving a certain confluence threshold metric. This information can then be automatically exported as a .CSV file for further analysis using a third party system if desired.

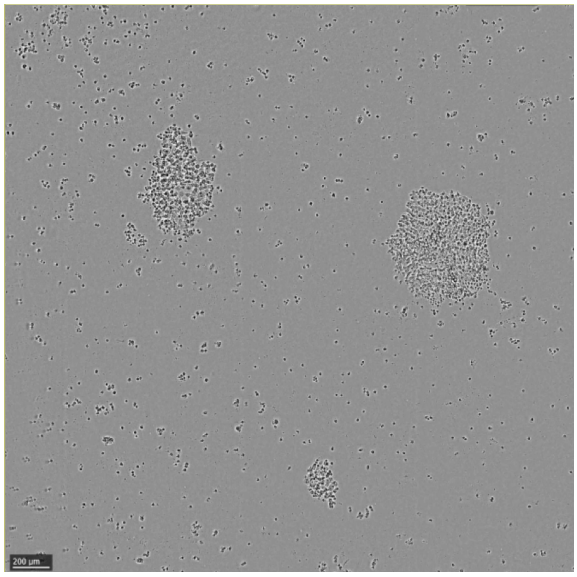
Software and software options

The software application for confluence determination is included as part of the standard Cell Metric package.

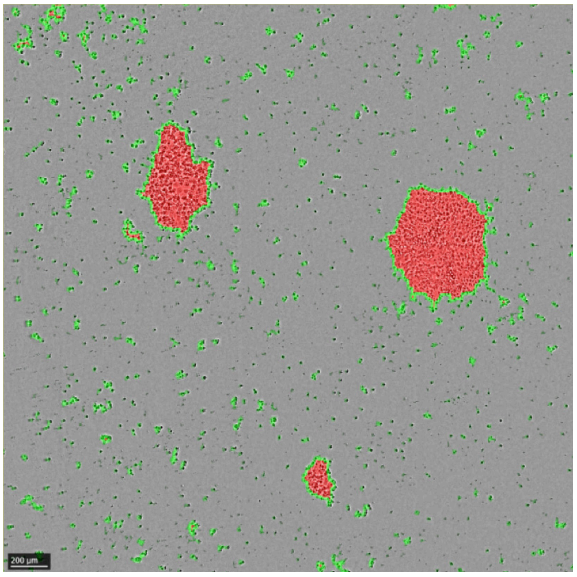
We offer various options for colony identification. Get in touch for more details.

Figure 6: A) Typical bright field image of colonies identified using software detection (note, image is from well A3 of the same sample plate as was shown previously in Figure 5); **B)** The colony indicated by a false colour overlay in red. The dead carpet of cells is highlighted in green.

A)



B)



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