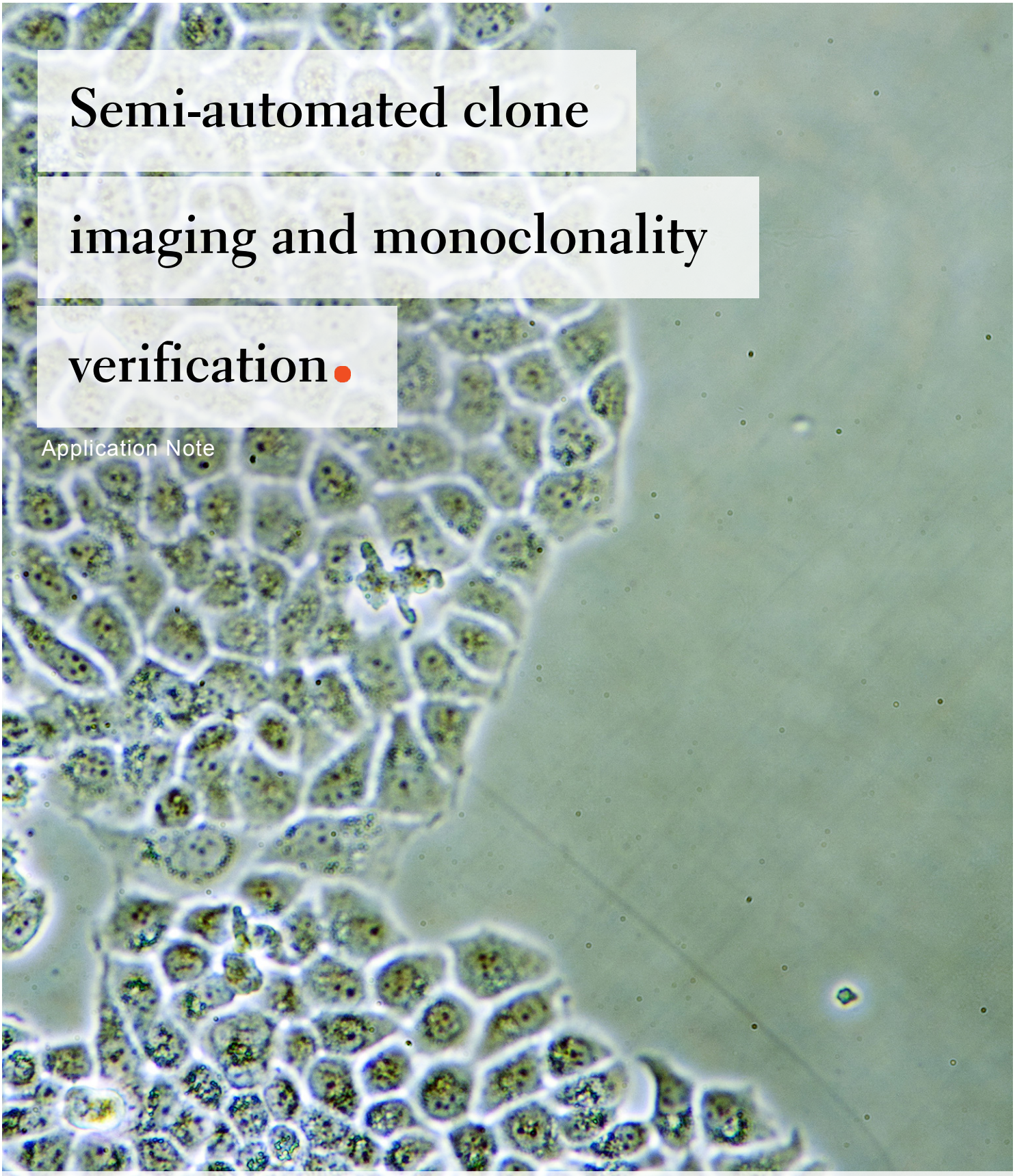
Semi-automated clone imaging and monoclonality verification.

Application Note



BRIGHTFIELD-BASED CELL IMAGING WITH THE SPARK® MULTIMODE READER

TECAN.

INTRODUCTION

For many cell biology applications – such as the identification and separation of high expression clones or knock-down candidates, and small-scale antibody or protein production – it is important to ensure that cell lines originates from a single cell, ie. they are monoclonal. This can be a major bottleneck, especially for academic laboratories, as dedicated automated microscopy systems are both very expensive and complex to operate, meaning verification of monoclonality is often performed by manual microscopy.

Tecan's new Spark® multimode reader platform has an integrated bright field imaging module that enables cell counting and viability analysis using disposable Cell Chips™. It enables label-free, real-time assessment of cell confluence in microplate wells, detecting the cell-covered areas and calculating the relative confluence ratios in 6- to 96-well plate formats. This imaging function is complemented by an intuitive, easy-to-use confluence analysis application in the reader's SparkControl™ software, offering reliable measurements with user-definable patterns (Figure 1). Designed for low to medium throughput applications, it can be used to perform confluence measurements in end-point or kinetic modes, with a total measurement time of less than 40 minutes for a 96-well plate. Depending on the assay requirements, the user can select different areas within the well (see Figure 2), allowing either one centered image per well, or a full-well picture created from multiple side-by-side images. The software also offers automatic well border detection to compensate for variations in the microplate dimensions.

This application note describes cost-effective cell counting and well imaging using the Spark to verify monoclonality in a 96-well plate format, based on automated image acquisition and semi-automated image analysis and data reduction.

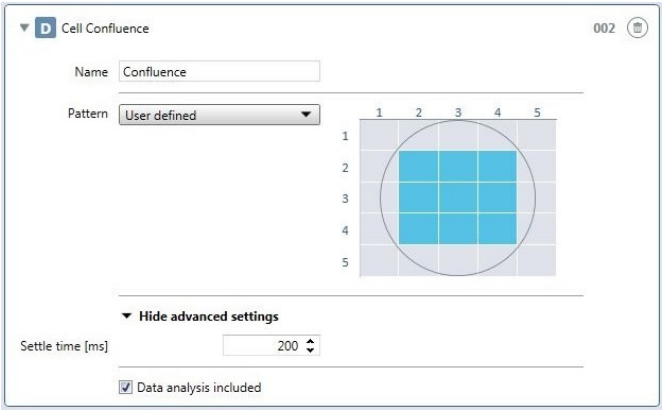


Figure 1: Confluence pane in SparkControl software.

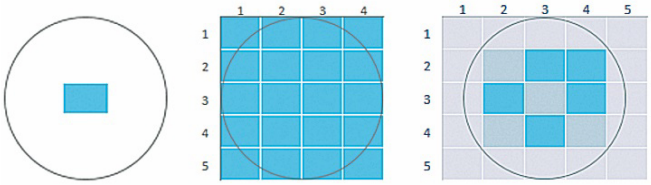


Figure 2: Options for confluence measurement patterns. For reliable identification of single cell clones, the Whole-well imaging function should be selected.

MATERIAL & METHODS

Cell seeding and growth

HEK293 cells grown to 70-90 % confluence were trypsinized and resuspended in normal culture medium, aiming for a concentration of 10 cells/ml. Cell concentration and viability was then determined using the Spark's cell counting module (1). 100 µl of cell suspension was dispensed into each well of a 96-well plate (flat bottom, cell culture treated). A positive growth control was included by seeding 1E+04 cells into one well of each plate. Cells were fed every 3-4 days, and were grown until they reached a confluence of approximately 20-30 % of the well (17 days).

Image acquisition and analysis

Image acquisition was performed four hours after seeding, once cells had slightly adhered to the bottom of the well, then once a week until cells reached the expected confluence. Whole-well imaging, Well border detection and Data analysis included were selected in the SparkControl software, with 20 images acquired for each well, then 'tiled' together. The use of Whole-well imaging and Well border detection ensured that single cells that had settled right at the edge of the well bottom were detected. When using the Data analysis included feature, the system saves both the raw and processed images.

To guarantee optimal cell viability during image acquisition, the reader's environmental control features were used to maintain a temperature of 37 °C and 5 % CO2 within the measurement chamber. Tecan's Humidity Cassette was also used to avoid evaporation of cell media. The system's Lid Lifter was used to automatically remove the Humidity Cassette lid (but not the culture plate's optically transparent lid) during measurements, helping to guarantee sterility throughout the experiment.

To identify cell growth, automatically analyzed whole-well images with a confluence of >10 % were manually screened on Day 17. These positive wells were then manually backtracked to Day 1, using the raw images to verify monoclonality. NOTE: Raw images without confluence assessment were used for manual assessments, as the high image quality of these images makes single cell identification easy.

RESULTS

Figure 3 shows a representative well for HEK292 where a single cell clone was identified.

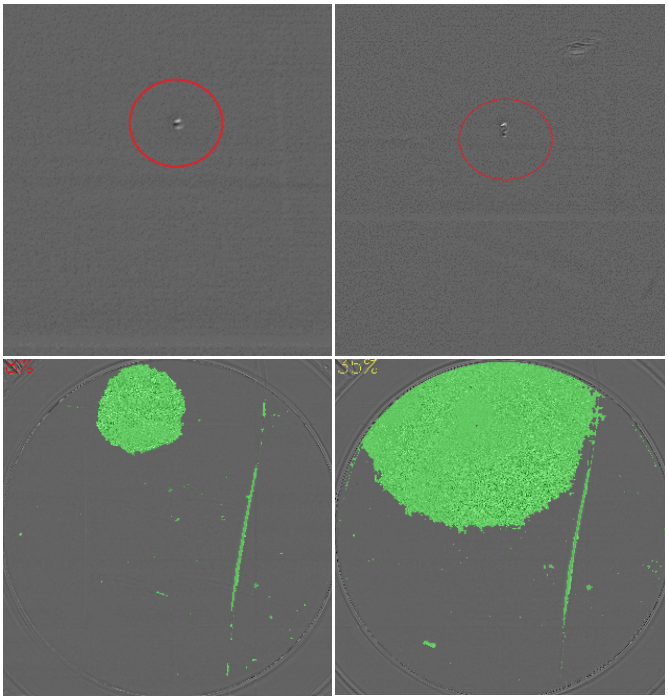


Figure 3: HEK293 cells – backtracking to a single cell clone after 17 days of cultivation.

DISCUSSION AND CONCLUSIONS

The results shown here clearly demonstrate the ability of the Spark's cell counting and well imaging module, in combination with the SparkControl software, to perform semi-automated clone imaging and monoclonality verification for low to medium throughput applications. The system enables reliable acquisition of images, with only a single autofocus error for more than 1,000 wells analyzed over the experimental period. The automatically determined confluence levels could be used to successfully identify cell colonies at Day 17, followed by visual inspection to determine if these cells resulted from a single cell clone. The success of this approach highlights that the Spark reader represents an ideal low-cost solution to eliminate the need for tedious manual monoclonality verification by microscopy.

REFERENCES

(1) Fast and accurate cell counting in a multimode microplate reader, 398570 V1.0. 12-2014

About the author

Dr. Christian Oberdanner is Product Manager at Tecan Austria. He studied molecular biology at the University of Salzburg with a strong focus on cell- and tumor biology during his PhD study. Christian started to work for Tecan Austria as external scientific consultant in 2005 and permanently joined the company in 2006. Since then he held the role of an Application Specialist in the research and development department as well as in the sales and marketing department and since 2015 of a Product Manager. Christian's priority within Tecan is the multimode microplate reader portfolio.

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