Application Note

LABEL-FREE CELL COUNTING IN iPSC PRODUCTION

Accurate label-free quantification of cell growth over time is crucial for successful maintenance, expansion and quality control of iPSCs. Here, TRI demonstrates how this can be achieved in a simple and cost-effective manner by utilizing the AI-based analysis capabilities of the VAIDR-system. TRI highlights several benefits of the method:

- ***Less effort - cell counting integrated in expansion workflow***
- ***Better quality control through unbiased AI-driven analysis***
- ***Robust starting point for subsequent differentiation***

The group of Sebastian Diecke at the Max Delbrück Center for Molecular Medicine (MDC) produces high-quality iPSCs for research into severe human disease. When cultivating iPSCs in a 2D format (flasks or multi-well plates), their precise proliferation status and speed are hard to quantify, as traditional analysis of microscopic images largely relies on staining agents, he says. These are incompatible with fragile live cells like iPSCs which need to be disturbed as little as possible.

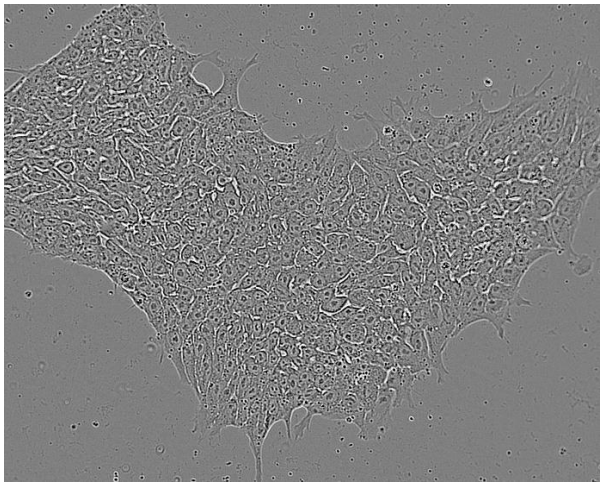


Figure 1: An iPSC colony imaged by the VAIDR microscope

However, knowledge about the growth of the cells helps the researcher to assess whether the conditions are optimal for maintenance and/or expansion. Variability in cell growth behavior is a critical quality control parameter which needs to be kept low to guarantee robust results of downstream processing like differentiation. The VAIDR system was developed specifically for this set of requirements and conditions.

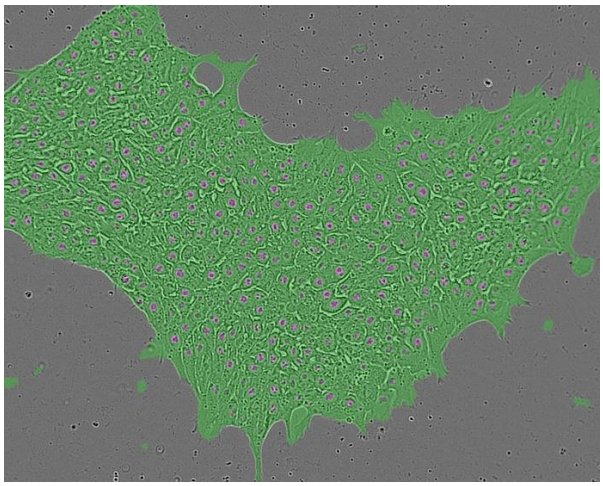


Figure 2: The same colony as in Fig.1, analyzed by the VAIDR software. Cell area is shown in green, cell centers in magenta.

VAIDR is an integrated system for automated microscopy and analysis. It combines universally applicable digital phase contrast microscopy with efficient

deep learning algorithms for unbiased pattern recognition and exposes these functionalities in a user-friendly interface which requires no training in data science.

VAIDR is uniquely helpful at quantifying a huge variety of cellular characteristics like phenotype, cell shape and structure, which makes it the perfect instrument to quantify cell proliferation parameters.

To illustrate the method, Diecke's lab cultured iPSCs in four different wells of a 6-well-plate for 96h. Two wells were initially seeded with 100k cells, and the other two wells were seeded with 200k cells. At 24h intervals, they acquired 49 images in each well. The VAIDR system was trained by hand-labeling to detect iPSC colonies as well as individual cell centers. Example images are shown in Fig.1.

The researchers used two parameters to characterize cell growth: Confluency was computed as the total colony area per image divided by the image area. To compute the cell count, they automatically determined the number of cell center objects in each image. Fig. 2 shows the confluency as a function time for the four wells and Fig.3 shows the corresponding cell count curves. The initial values for all curves reflect the seeding density, as expected. Cell growth starts after the 48h time point and the high seeding density leads to a higher initial growth rate, which remains roughly constant for the duration of cultivation. At the same time, the low seeding density curves show a smaller but increasing growth rate.

Having **unbiased control** over complementary cell growth parameters like confluency and cell count offers several benefits, TRI notes: Anomalous behavior can be detected early, e.g., one of the parameters or their relation deviates from the norm. Additionally, having the cell count integrated into the expansion QC allows for a **reduction in effort** in the transition to the next step: Before differentiating IPSCs into the desired cell type, a precise cell count Is required. Using VAIDR can obviate the need for additional cell counting steps.

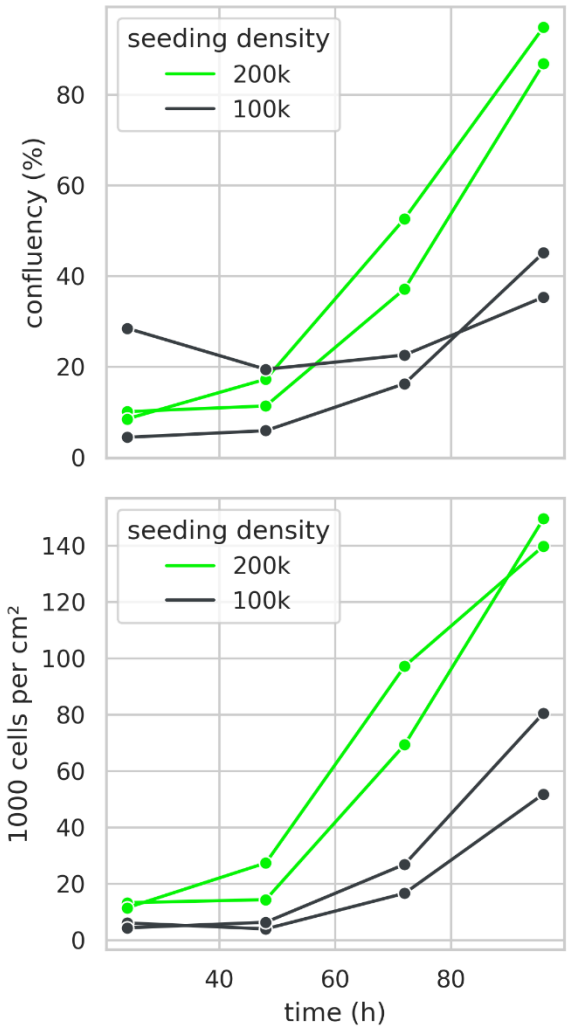
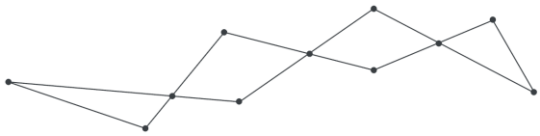


Figure 3: iPSC confluency (top) and cell count (bottom) as a function of cultivation time



In summary, TRI has introduced a label-free method for quantification of iPSC growth under production conditions, in the context of a long-term collaboration with Sebastian Diecke's lab. The method can be easily integrated into iPSC production processes and does not require prior training in image analysis.

For more information on Sebastian Diecke's research at the Max Delbrück Centrum, visit www.mdc-berlin.com/pluripotent-stem-cells

To learn about VAIDR, visit www.vaidr.de