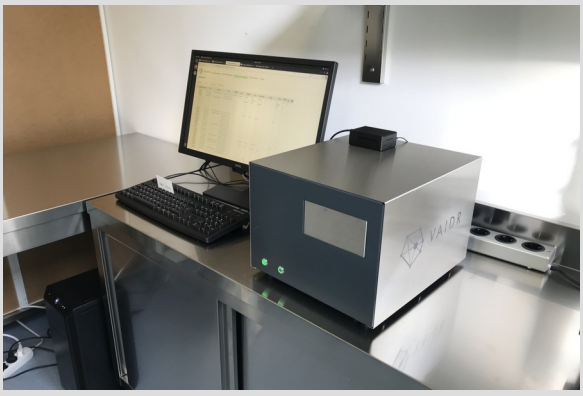
Application Note

# Towards a New Standard in Viability monitoring: AI-assisted Imaging Makes Cell Health Quantification More Sensitive and Efficient

In cell cultivation for in-vitro experiments or for cell therapies, consistent cell health is a critical success factor. It depends on a multitude of factors and small fluctuations in culture conditions may affect it at any point in time during cultivation. It is therefore desirable to establish a tight control on cell health during production. Such a routine application must be as non-invasive and cost efficient as possible. Here, we demonstrate how a sensitive, label-free cell health quantification method can be easily established for live THP-1 cells using the VAIDR system. In contrast to established methods for cell viability measurement, we show that even subtle, early signs of diminishing cell health can be quantified reliably, opening the path towards overall improved performance in manufacture of high-value cell products.



VAIDR combines streamlined microscopy with AI-assisted image analysis. 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.


acCELLerate is a specialist for large-scale production and cryopreservation of highly functional cell lines in an Assay Ready Cell condition. Assay Ready Cells are cryopreserved at a highly functional state and can be used instantly after thawing like a reagent. There is no need to further expand or passage the cells beforehand. The high quality of pre-qualified assay ready cells helps to increase the precision and reliability of any cell-based assay.

## Cell Health Quantification is Critical

Efficiently and reliably detecting the health state of cells is essential in reproducible research and production of high value cell products. Failing to exercise consistent control over the health state will inevitably lead to unreliable research results and low quality products. However, established methods can be expensive in terms of reagents and labor and may require destruction of the sample. Additionally, traditional cell viability measurements may not be sensitive enough, as subtle signs of reduced cell health which are relevant to quality and consistency often manifest themselves long before a cell actually dies.

Here, we introduce an improved approach to achieve this goal using VAIDR, an automated imaging and AI-based analysis system. We compare the results to customary methods of fluorescence-based flow cytometry (I), conductivity based cell viability counter (CASY) (II) and visual assessment using a Neubauer chamber (III).

We used the THP-1 suspension cell line as a model system and investigated one batch directly after thaw and 24h later as a classical recultivation setup.

For the CASY count, cells are suspended in CASYton and are counted using Electronic Current Exclusion (ECE) and Pulse Field Analysis. When using the flow cytometer the cells are first stained with PI. PI enters dead cells that have compromised membranes and stains the cells with red fluorescence. For a Neubauer analysis the cell suspension is diluted with Trypan blue. Trypan blue passes through the damaged membrane of dead cells. Under light microscopy analysis, only dead cells have a blue color. All cells in the chamber have to be then counted by hand.

## VAIDR Makes Expert Knowledge Quantitative

THP-1 cells display a distinct morphological phenotype which correlates with their state of health: A round shape and smooth borders indicate a healthy state whereas an irregular shape and rough borders indicate an unhealthy state.

Such differences are easily recognized by a trained human expert. However, building a quantitative, objective, and comprehensive QC process on human image evaluation is somewhere between costly and prohibitive. VAIDR allows the implementation of the trained analysis of the cell morphology in a simple and cost efficient way due to the automated data processing

To develop an automated method for cell health quantification, first THP-1 cells were sub optimally frozen, in order to cause a decrease in the cell viability during the post-thawing cultivation process. Those cells were imaged using the VAIDR microscope, which generates digital phase-contrast images. A cell-culture expert then labeled 3 images using the intuitive user interface of the VAIDR system. The labeling process essentially consists of painting the different phenotypes in specific

colors[1]. In this case, cells were labeled as optimal, damaged or clumped. These labels were then passed to the system to train a classification model.

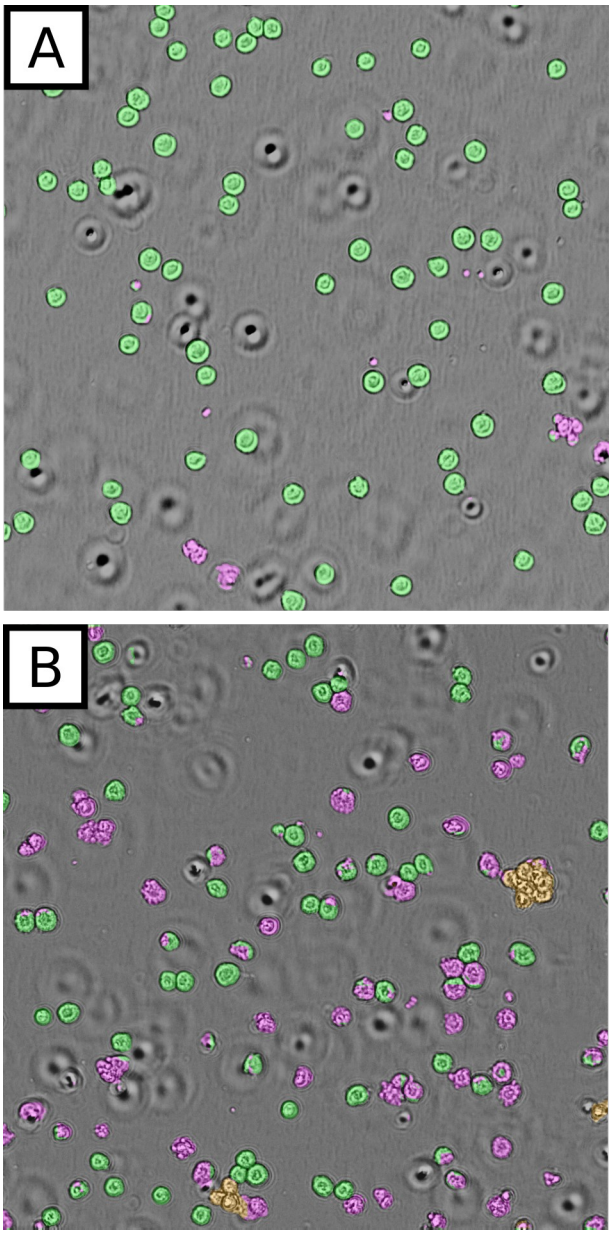



*Figure 1: Images of THP-1 cells analyzed by VAIDR. Green designates the optimal (healthy) phenotype, magenta indicated damaged and yellow clumped cells. (A) Directly after thawing (B) 24h after thawing.*

The model was then used to predict the cell health state for all images acquired from the cell culture flasks. Some of the automatically analyzed images were used to validate the successful training.

The model distinguishes the optimal, damaged and clumped phenotypes (Figure 1). In contrast to human labeling, the automatic labeling even detects parts of cells which exhibit more of one or the other phenotype, allowing for an even finer distinction and quantification than the original human-labeled data would suggest.

*Table 1: Quantitative results of cell viability reference methods and VAIDR analysis*

| | | Thaw | Thaw + 24h |
|---|---|---|---|
| Viability (%) | CASY | 96.0 | 87.1 |
| | Flow Cyt. | 99.3 | 81.9 |
| | Neubauer | 97.0 | 71.3 |
| VAIDR (%) | Optimal | 77.2 | 44.5 |
| | Damaged | 20.8 | 50.2 |
| | Clumped | 2.0 | 5.3 |

To evaluate the results quantitatively, the relative areas covered by optimal, damaged and clumped cells were analyzed for both samples. The results are shown in Table 1. The 24h cultivation period drastically increases the fraction of damaged and clumped cells. A more detailed look into the VAIDR results is provided by Fig. 2.

## VAIDR is More Sensitive

Table 1 also shows the results of the reference methods. In all cases, the viability rates are much higher than the percentage of optimal

1 For a more detailed description of the training process, refer to our earlier application note: https://www.vaidr.de/wp-content/uploads/2022/03/appnote-luhmes-vaidr.pdf

cells in the VAIDR result. Since the automated labeling by VAIDR agrees with the manual labeling by the cell expert, this indicates that visual quality evaluation measures a different quantity from traditional cell viability, although both undoubtedly measure quality. Therefore the comparison must remain qualitative.

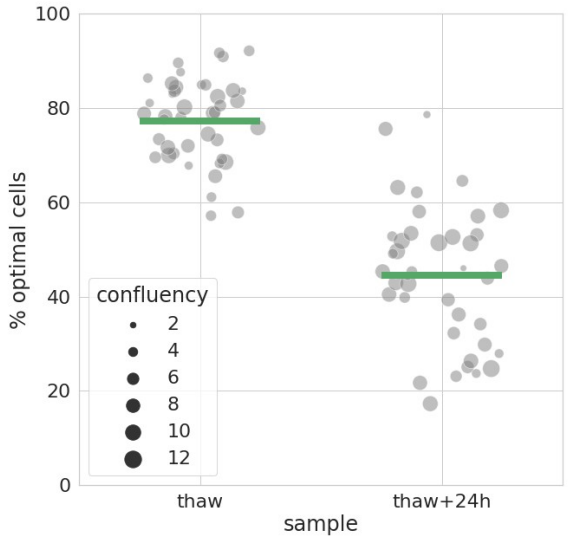



*Figure 2: Total percentages of optimal cells are shown by the green bars for both samples and the values of individual image fields are shown by gray circles. The fraction of cell-covered area (confluency) is indicated by the marker size.*

The mechanisms for classifying a cell as not viable in the case of the cell viability counter and flow cytometry are based on membrane permeability. If the cell membrane has become so leaky that these methods register a non-viable cell, this cell is already severely damaged. The image-based approach by VAIDR gives a more distinctive result and, interestingly, shows that the human expert's definition of a healthy cell (which has been learned by VAIDR) is much stricter than the traditional viability methods. This finding highlights the potential of using VAIDR in quality control: While the verdict of a human cell-culture expert is rightly considered the superior metric, it is (a) usually only qualitative, (b) somewhat subjective and (c), as we have shown here, often not in agreement with the more indirect, customary viability measurements. And most importantly, human cell-culture expert's time is too valuable to spend on large-scale image evaluation. With VAIDR, a notion of cell health that is directly linked to the human expert's assessment is turned into a quantitative, objective and reproducible measurement.

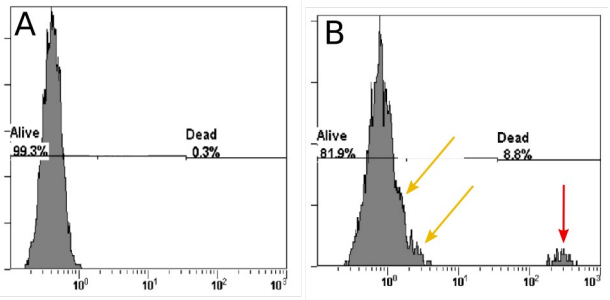
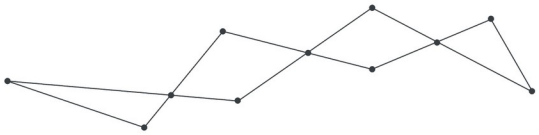



*Figure 3: Fluorescence intensity histograms from the flow cytometry result. (A) Directly after thawing, (B) 24h after thawing. Yellow arrows indicate cells with slightly increased fluorescence signal and red arrow indicates the high-intensity peak from dead cells.*

Hints of reduced cell health in the 24h sample are visible in Fig. 3 as shoulders on the right flank of the low intensity peak (yellow arrows) in the flow cytometry result, but these are completely separated from the positively PI labeled cell population (red arrow) and are therefore not quantified as dead cells. We suspect that cells contributing to such shoulders would likely also show a sub-optimal visual morphology. This could be an indication that analysis using VAIDR could allow early detection of changes in cell viability Therefore, we see significant potential

in quality improvement and cost-savings, as the earlier flagging of issues can be used to drive better decisions in the production process.



## Conclusion

In summary, we have shown an example of a successfully developed, non-invasive cell health quantification method using the VAIDR system. No specific data science knowledge was required to set up the analysis, as all of the deep-learning and data-logistics capabilities are easily accessible through the user interface. The VAIDR method works directly with the cell culture vessel and requires no further treatment of the sample, which differentiates the method from traditional approaches. The most striking difference to customary methods, however, is the higher sensitivity with which VAIDR can detect signs of reduced cell health which may lead to cell death at a later stage. Mimicking the visual perception of a human cell-culture expert, VAIDR efficiently quantifies the relevant biological effects for most objective decisions. We conclude that using VAIDR is an additional quality measure in cultivated cell handling to optimize downstream results.

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