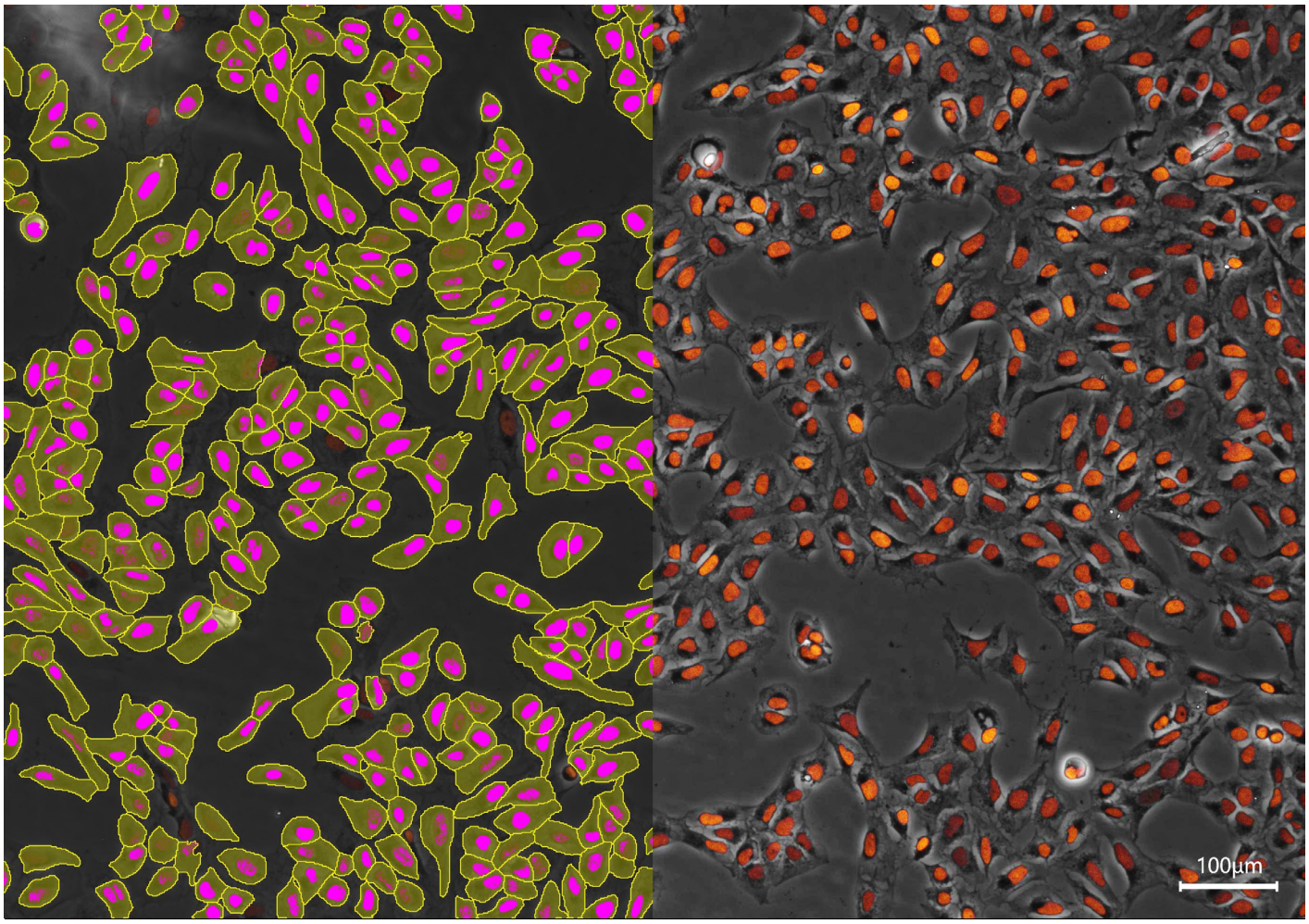
From Eye to Insight

Leica MICROSYSTEMS

LEVERAGING AI FOR EFFICIENT ANALYSIS OF CELL TRANSFECTION

How to optimize your transfection efficiency measurements for 2D cell culture with AI



AI-based transfection analysis (left) of U2OS cells which were transfected with a fluorescently labelled protein. A fluorescence image of the cells (right) is also shown. The analysis and imaging were performed with Mateo FL.

Authors

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Introduction

In the realm of cell biology and molecular research, the integration of artificial intelligence (AI) has emerged as a transformative force, offering novel solutions to longstanding challenges. Accurate transfection efficiency measurements for 2D cell culture studies are paramount for unraveling critical cellular mechanisms and advancing scientific understanding [1-3]. Ensuring high transfection efficiency is crucial, as it guarantees the optimal expression levels of the proteins of interest, indispensable for a spectrum of scientific experiments, including live cell imaging and protein purification. Traditional methods reliant on manual estimation are plagued by inconsistencies, leading to unreliable results.

This app note focuses on the significance of precise measurements in 2D cell culture workflows, shedding light on the pitfalls and challenges associated with existing techniques, particularly when labeling strategies fall short. By harnessing the power of AI, researchers can overcome these hurdles, achieving enhanced reliability and efficiency in transfection studies.

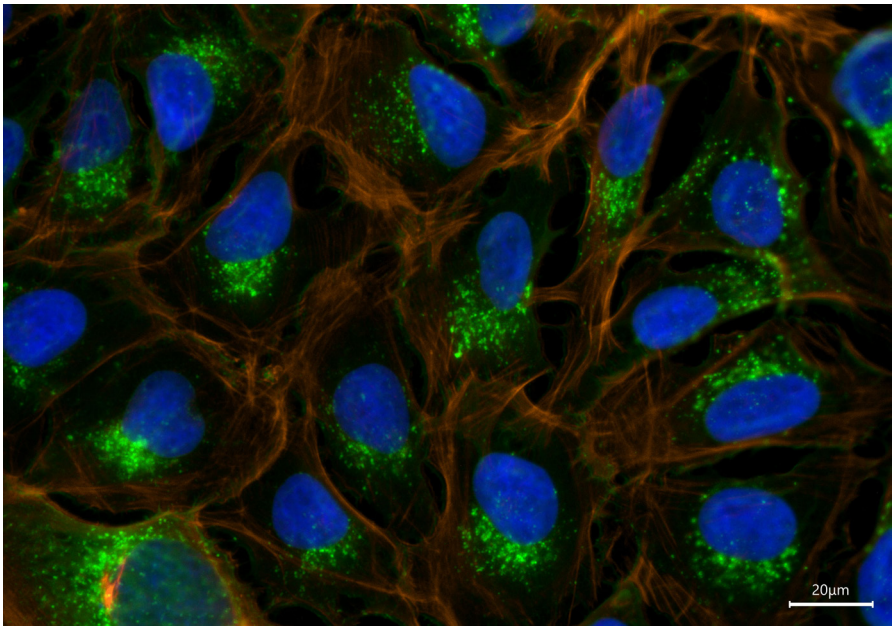


Fig. 1: Image of fluorescent U2OS cells labelled with DAPI (blue), Mitotracker (green), and Actin (orange).

AI algorithm development

The heart of achieving precise transfection efficiency quantification lies in the strategic development of AI algorithms. These algorithms, whether pre-trained or specifically tailored through training, play a pivotal role in deciphering intricate cellular dynamics. By considering factors such as cell morphology, fluorescence intensity, and background noise, these intelligent systems navigate the complexities inherent in 2D cell culture studies. Pre-trained algorithms bring the advantage of leveraging prior knowledge, while custom training allows adaptation to unique experimental conditions. The incorporation of these algorithmic considerations ensures a nuanced understanding of cell transfection dynamics, leading to more accurate and reliable measurements.

Comparison of transfection efficiency data with t-test analysis

The cell-transfection-efficiency values obtained with Mateo FL using AI and then compared with the best-estimate value are shown in Table 1 and 3 below. The best estimate refers to the most accurate determination by humans of the percentage of cells with the fluorescent marker. The comparison was done with percent difference.

Cell transfection efficiency: Mateo FL vs. best estimate

Test round	Mateo FL	Best estimate	% difference Mateo FL & best estimate value
1	45	50	10
2	61	67	8.96
3	68	70	2.86
4	60	66	9.09
5	70	74	5.41

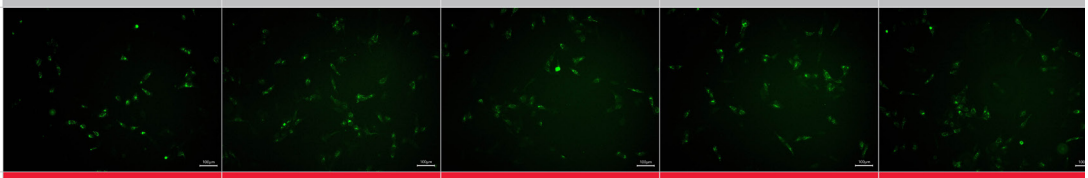
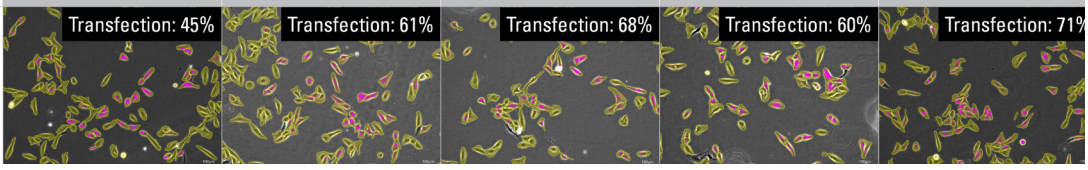
Table 1: Comparison of transfection-efficiency values from Mateo FL and best estimate.

The cell-transfection-efficiency values for Test round 3 obtained manually and then compared with the best-estimate value are shown in Table 2 below. The comparison was done via statistical analysis using a t-test. There is a significant difference between the transfection-efficiency values for manual determination and best estimate.

Cell transfection efficiency: manual vs. best estimate

Test round 3 Tester	Manual	Best estimate	T- test comparison manual & best estimate value
A	40	70	t value = -17
B	45		Degrees of freedom (df) = 3
C	46		p value = 0.0004
D	40		$p < 0.05$
Average	43		**Significant difference**
Standard error of mean (SEM)	2		

Table 2: T-test comparison of transfection-efficiency values from manual determination and best estimate.

Test round	1	2	3	4	5
Test image					
Best estimate*	**50**	**67**	**70**	**66**	**74**
Tester A	60	78	40	70	74
Tester B	55	80	45	64	81
Tester C	39	56	46	50	63
Tester D	40	55	40	54	59
AI result	**45**	**61**	**68**	**60**	**71**
Gap Tester A vs. AI result (%pt.)	-33%	-28%	41%	-17%	-4%
Gap Tester B vs. AI result (%pt.)	-22%	-31%	34%	-7%	-14%
Gap Tester C vs. AI result (%pt.)	13%	8%	32%	17%	11%
Gap Tester D vs. AI result (%pt.)	11%	10%	41%	10%	17%
AI image					

* Refers to the most accurate determination made by humans regarding the percentage of cells that successfully incorporate the fluorescent marker.

Table 3: Summary of results from manual and AI cell-transfection-efficiency determination which are compared to best-estimate values.

Seamless integration of AI methodologies into upstream workflows

Beyond optimizing transfection efficiency measurements, the impact of AI extends into upstream workflows, fundamentally reshaping traditional methodologies in protein purification, isolation, extraction, microscopy imaging (widefield and confocal), and flow cytometry. AI-driven advancements empower users to streamline these processes, enhancing efficiency and reliability. The integration of AI methodologies into upstream workflows not only accelerates experimental timelines, but also enhances the precision and reproducibility of critical steps in cellular research.

For instance, AI algorithms can predict optimal conditions for protein purification based on historical data, reducing trial and error in the laboratory. In imaging, AI enables automated analysis of confocal and widefield images, expediting the extraction of meaningful information from complex datasets.

Future perspectives

The ongoing development of more sophisticated AI algorithms, driven by machine learning and deep learning approaches, promises heightened precision in cellular analyses. Future applications may include the ability to predict optimal transfection protocols based on real-time experimental feedback, ushering in a new era of adaptive experimentation. Furthermore, the convergence of AI with other cutting-edge technologies, such as single-cell omics and advanced imaging modalities, is set to reveal unprecedented insights into cellular behavior. Enhanced predictive models, capable of deciphering intricate cellular responses, will facilitate the discovery of novel pathways and mechanisms, accelerating drug development and personalized medicine.

The rapid pace of innovation in AI demands a proactive approach to staying informed, embracing new tools, and fostering interdisciplinary collaborations. As the field continues to evolve, the integration of AI methodologies into cell culture studies promises not only increased efficiency, but also the potential for discoveries that will shape the future of cellular and molecular research.

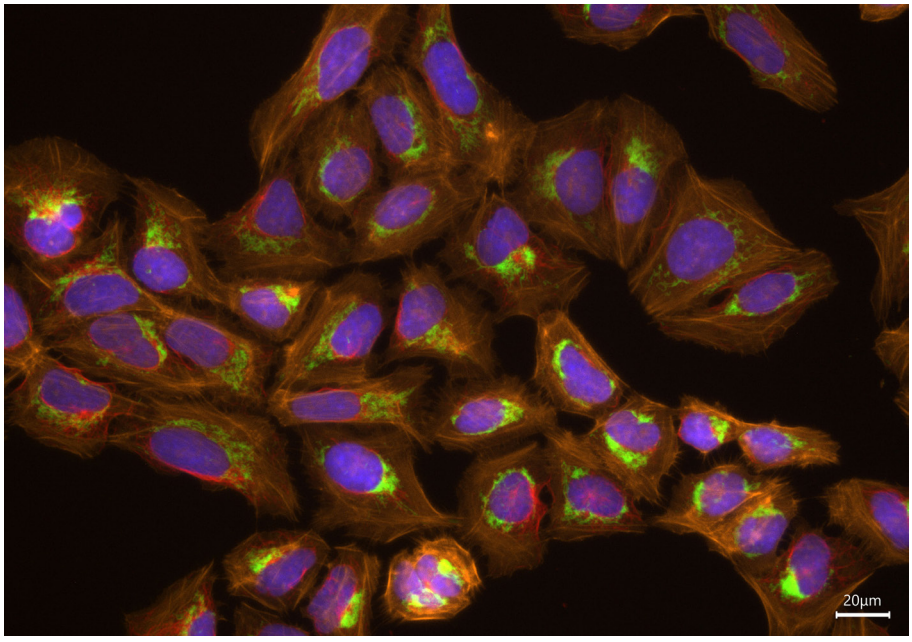


Fig. 2: U2OS cells stained with 405 Hoechst33342 DNA, AF488 Tom20, SPY 555 Actin, and Atto 647 Tubulin.

Conclusion

The integration of AI has transformed transfection efficiency measurements in cell culture studies, addressing challenges of manual methods. AI algorithm development ensures nuanced understanding, considering factors like cell morphology and background noise. Seamless integration into upstream workflows, from protein purification to imaging and flow cytometry, enhances efficiency and reproducibility. Future perspectives highlight evolving AI applications, offering adaptive experimentation and deeper insights into cellular behavior. These exciting prospects should encourage users to stay abreast of these evolving technologies for enhanced transfection efficiency analysis in 2D cell culture.

References

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