



EAP4EMSIG –An AI-powered Pipeline for Real-time Event-based Experiment Automation in Microfluidic Live Cell Imaging

Microorganisms hold great potential to advance biotechnology, medicine, and environmental science. In particular, Microfluidic live-cell imaging (MLCI) allows biotechnologists to study microbial growth dynamics at the single-cell level. The current technology typically employs a two-step approach: first, high-quality image data is acquired through high-throughput experimental setups, and only then analyzed to extract quantitative and qualitative insights. While this approach has been instrumental in generating valuable information, it inherently limits the ability to respond dynamically to events as they occur. The lack of real-time analysis and reactivity hinders leveraging immediate insights, which are crucial for making timely adjustments during experiments and understanding transient phenomena efficiently. This limitation underscores the need for innovative solutions that enable real-time data processing and decision-making.

In this work, we present an Artificial Intelligence (AI)-based Experiment Automation Pipeline for Event-Driven Microscopy in Smart Microfluidic Single-Cell Analysis (EAP4EMSIG). This pipeline addresses the limitations of traditional two-step approaches in MLCI by enabling real-time data acquisition, analysis, and decision-making. EAP4EMSIG harnesses the potential of AI by incorporating deep learning-based modules for real-time autofocus and segmentation. The autofocus module predicts focus offsets from single microscope images with sub-micron accuracy (Mean Absolute Error: $0.2013 \pm 0.07 \mu\text{m}$) and achieves inference times of less than 50 ms. The real-time segmentation module incorporates state-of-the-art methods, including Cellpose 3, which achieves a Panoptic Quality of 93.58%. Notably, the distance-based method demonstrates the fastest performance, with inference times below 50 ms, rendering it highly suitable for real-time applications in dynamic experimental settings. Additionally, the pipeline includes a real-time data analysis dashboard that provides actionable insights during ongoing experiments.

By integrating AI-driven autofocus and fast segmentation, EAP4EMSIG advances high-throughput microscopy automation, reducing manual intervention and improving reproducibility. The pipeline transforms MLCI into a proactive system, enabling real-time event detection and dynamic control, while enhancing imaging quality and thereby improving the efficiency of MLCI.

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