

EAP4EMSIG - Experiment Automation Pipeline for Event-Driven Microscopy to Smart Microfluidic Single-Cells Analysis

Nils Friederich^{1,2,*}, Angelo Jovin Yamachui Sitcheu^{1,*},
Annika Nassal^{1,2}, Matthias Pesch³, Erenus Yildiz⁴,
Maximilian Beichter¹, Lukas Scholtes⁴, Bahar Akbaba¹,
Thomas Lautenschlager¹, Oliver Neumann¹, Dietrich Kohlheyer³,
Hanno Scharr⁴, Johannes Seiffarth^{3,5,#}, Katharina Nöh^{3,#},
Ralf Mikut^{1,#}

¹ Institute for Automation and Applied Informatics (IAI)

² Institute of Biological and Chemical Systems (IBCS)
Karlsruhe Institute of Technology

³ Institute of Bio- and Geosciences (IBG-1)

⁴ Institute for Data Science and Machine Learning (IAS-8)
Forschungszentrum Jülich GmbH

⁵ Computational Systems Biology (AVT-CSB)
RWTH Aachen University

*Contributed equally

#Supervised equally

Abstract

Microfluidic Live-Cell Imaging (MLCI) generates high-quality data that allows biotechnologists to study cellular growth dynamics in detail. However, obtaining these continuous data over extended periods is challenging, particularly in achieving accurate and consistent real-time event classification at the intersection of imaging and stochastic biology. To address this issue, we introduce the Experiment Automation Pipeline for Event-Driven Microscopy to Smart Microfluidic Single-Cells Analysis (EAP4EMSIG). In

particular, we present initial zero-shot results from the real-time segmentation module of our approach. Our findings indicate that among four State-Of-The-Art (SOTA) segmentation methods evaluated, Omnipose delivers the highest Panoptic Quality (PQ) score of 0.9336, while Contour Proposal Network (CPN) achieves the fastest inference time of 185 ms with the second-highest PQ score of 0.8575. Furthermore, we observed that the vision foundation model Segment Anything is unsuitable for this particular use case.

1 Introduction

What are microbes? Microbes, also known as microorganisms, are a group of tiny living organisms that are invisible to the naked eye. This group includes bacteria, archaea, fungi and protists [5]. Microbes are present almost everywhere on Earth, from harsh environments such as hydrothermal vents to the human body, where they outnumber human cells by a factor of around 1.3 [54]. Despite their tiny size, microbes play crucial roles in various ecological and biological processes, making them essential for life on Earth [30].

Why are microbes relevant? Microbes are relevant for several reasons. The first is ecological balance, where microbes are essential in the nutrient cycle, decomposing organic matter and contributing to soil fertility [57]. They are crucial for the carbon, nitrogen and sulfur cycles that sustain life on Earth [27]. Second, in human health, the human microbiome aids digestion, produces essential vitamins and protects against pathogenic microbes [38]. Disruptions in the microbiome can lead to health issues such as infections, obesity and autoimmune diseases [7, 29]. Finally, in the context of industrial applications, microbes are harnessed in biotechnology, pharmaceuticals and agriculture. They are used to produce antibiotics, biofuels and fermented foods [25]. Microbial enzymes are also crucial in many manufacturing processes [46].

Why is research on microbes essential? Research on microbes is important due to their impact on health, industry and the environment. Understanding microbial behavior, genetics and interactions can advance all three areas. In

medical science, it is crucial to study pathogens to help develop vaccines and treatments for infectious diseases [2]. Microbe research can potentially reveal new therapies for chronic diseases [3]. In environmental protection, microbes can be used in bioremediation to clean up oil spills and toxic waste [18]. Therefore, understanding microbial ecosystems can help conservation efforts and combat climate change. In biotechnology, microbial research can lead to the development of new applications, such as using microbes to produce valuable compounds, e.g., insulin or biodegradable plastics [36].

Why is the segmentation of microbes relevant? While some biological analysis is possible at the macroscopic level, other results can only be obtained by studying organisms at the microscopic single-cell level. MLCI particularly enables an understanding of single-cell growth and growth heterogeneity due to very small volumes. For example, the effect of antibiotic concentrations on organism performance can be analyzed through such experiments. Understanding the dynamics of microbes at this single-cell level therefore requires accurate and precise automated cell segmentation, as large amounts of data acquired using automated microscopy must be analyzed to obtain relevant results. The segmented data can then be used to make statements about the organism's growth as a function of various other factors.

What is the challenge in microbe research? MLCI experiments with microbes are usually not carried out on a single colony but in parallel on thousands. To achieve this, the microfluidic device is infused with a cell suspension and cells are randomly seeded into the growth chambers, where they remain trapped. Optimally, a microbial colony grows in each chamber. In a standard growth experiment, seeded cells grow until the chamber is filled with densely packed cells, which can be 1000 ends, which marks the end of the experiment. Subsequent experiment examination requires analyzing thousands of colonies in parallel, with thousands of microbes in some cases. Each chamber must be manually assessed to determine whether it meets the experiment's objectives, even as some chambers become irrelevant as the experiment advances. This process is extremely time-consuming, costly,

strenuous, monotonous and nearly impossible. Therefore, automated and intelligent processing, analysis and experiment planning are required.

How does this paper address this challenge? In this paper, we introduce the EAP4EMSIG, designed to automate and intelligently execute MLCI experiments, during which the human expert specifies settings, monitors progress and intervenes only to address any issues that may arise. We explain the concept of the pipeline and its eight primary modules. To achieve this, a literature review (see Section 2) and an extensive description (see Section 3) of each module are provided.

Since real-time data evaluation, inference and incorporation into the experimental design are central parts of our entire Experiment Automation Pipeline (EAP) pipeline, our work will compare initial results. We will compare the results related to the Average Precision (AP) [28] score, PQ [23] score and inference time of four SOTA methods from the task, domain and foundation areas, using a large publicly available microbial benchmark dataset [51,52] (see Section 4). For this purpose, their zero-shot abilities and their real-time capability will be analyzed and investigated to determine which models are suitable for retraining. Additionally, we will evaluate whether using a foundation model potentially leads to better results through improved generalization.

2 Related Work

Experiment Automation Pipelines. Various EAP tools have been developed, ranging from local standalone projects [10] to cloud-based tools [34]. Some methods focus on automating the data analysis part [17,37], others focus on automating the data acquisition part [43], particularly on microscope control [40, 42] and event-based image acquisition [6,33]. However, very few generic tools for full experiment automation have been proposed due to the complexity of combining the experiments' software, hardware and biological components. One example is the PYthon Microscopy Environment (PYME)¹ open-source

¹ <https://www.python-microscopy.org/>

package, which offers data acquisition, processing, exploration and visualization modules. PYME is, however, only tailored for super-resolution techniques. Another example is Cheetah [39], a Python library that automates real-time cybergenetic experiments. It offers limited microscope control capabilities and relies on one specific image segmentation method, i.e., U-Net [44].

Recently, the EAP tool MicroMator [16] has emerged, strongly aligning with our goal. Similarly to the idea of smart futuristic microscopy depicted in [4] and [41], MicroMator supports reactive microscopy experiments. The developed open-source package is modular, extendable and adjustable for several experiments. However, they offer limited image analysis possibilities and no tool to improve the image analysis results. Moreover, the software seems not to be actively used and maintained.

In summary, while many tools exist that each contribute to a step towards fully EAP, no tool, to the best of our knowledge, provides a complete, modular and extendable pipeline that manages event-based data acquisition, analysis and reporting.

Segmentation. Deep learning-based segmentation methods have recently emerged as they are often faster, more accurate, and precise than traditional methods [14], given sufficient training data availability [12].

A method with pixel-wise segmentation is required to obtain all the information needed for event detection in the context of microbes. To allow the extracted data to flow directly in the EAP during the experiments, this method must be fast enough, accurate and precise to enable real-time processing [31]. Therefore, objects can be segmented, for example, with generalized methods like the SOTA vision model Segment Anything [24], so-called foundation models, which attempt to recognize all objects correctly, e.g. in the context of segmentation. Although these methods can recognize many diverse objects, they may have limitations in precision for a single use-case [22]. To overcome this problem, there are also SOTA domain-specific biomedical methods like CPN [58] and StarDist [50] or task-specific-models like Omnipose [9].

With the wide variety of models available, selecting the most appropriate one for a given task remains a significant challenge. Currently, this selection is still performed manually. However, solutions that aim to automate this selection process are being proposed. [19, 35, 55] investigate image similarity metrics to select the best model for a given task.

Experiment Database. MLCI experiments produce vast amounts of data. This data and associated metadata must be stored and managed for subsequent analysis and reporting. In the context of EAP4EMSIG, the data management tool must support the FAIR data management principles as depicted in [59].

For our work, the most suitable tool is Open Microscopy Environment Remote Objects (OMERO) [1], an open-source tool for managing, analyzing and visualizing microscopy images and their metadata. It offers a centralized, secure and scalable solution for handling diverse imaging data types, facilitating collaboration and data sharing among entities. Compared to other SOTA data management tools, OMERO provides advanced visualization tools and supports integration with other image analysis software [49].

Semi-Automated Data Annotations. To train the segmentation methods, corresponding training data are crucial. Annotating this data is typically time-consuming, so semi-automated segmentation tools like KaIDA [48] or ObiWan-Microbi [53] are often employed in biomedical use cases [24, 47, 60]. This process involves training a network with a small amount of manually annotated datasets and manually refining the network's predictions by a human annotator on new unannotated datasets.

AI-ready Image with Ground Truth Cell Simulation. A significant challenge in applying Deep Learning (DL) techniques is the need for labeled data for training and validation. Particularly in cell instance segmentation tasks, pixel-exact masks that accurately distinguish individual cells from the background are essential. Due to the high costs needed to generate such labeled data [21], cell simulators have been developed [26, 56]. Their aim is to create realistic, controlled and reproducible cellular models that accurately reflect biological

processes. For bacterial microcolony ground truth generation, particularly in the context of phase contrast microscopy, the cell simulator CellSium [45] emerges as the suitable tool in this work. It is an agent-based, highly customizable and versatile simulator that produces data for different use cases.

Module Interaction. Given the complexity of integrating software, hardware and biological components in laboratory experiments, a suitable architecture is required. This architecture must be robust, understandable, modular and most importantly extendable due to the uniqueness of each laboratory experiment. For our EAP, we currently use Robot Operating System (ROS) [32], an open-source framework primarily for developing robot software.

ROS provides a modular building architecture based on the central notion of nodes. Each node represents a functional unit and can exchange messages with another node, particularly in an event-based manner. Hence, ROS is very suitable for real-time tasks in various fields, such as in [20]. Nevertheless, due to the high complexity of installing and maintaining ROS as well as its dependency bugs [15], just very few approaches use it. The closest to ours is Archemist [13], an experiment-automating system for chemistry laboratories.

An alternative to ROS which is currently being investigated for our EAP is Dataflow-Oriented Robotic Architecture (DORA)², a framework designed to ease and simplify the development of AI-based robotic applications. To the best of our knowledge, DORA is very new and has not been used for experiment automation tasks yet. It provides low-latency, composable and distributed dataflow capabilities. Applications are organized as directed graphs, often referred to as pipelines. Although it is much faster than ROS, it is still unstable and has a rather smaller community.

3 Methodology

To fill the noted gaps, we propose a new EAP approach, which is fully described module-by-module in this section. As shown in Fig. 1, our system consists of

² <https://dora-rs.ai/>

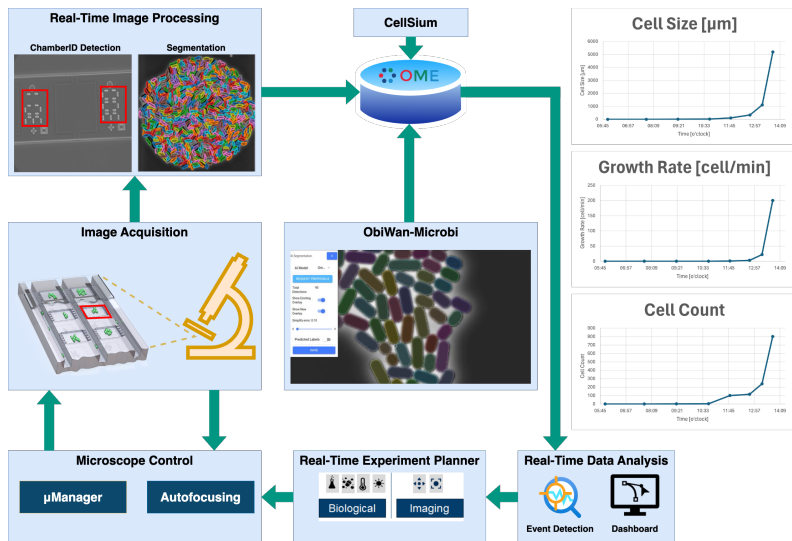


Figure 1: EAP4EMSIG visualization. The pipeline consists of eight modules, represented by the light blue boxes and the OMERO database, arranged in a cyclical process. The microbial images in the figure come from dataset [51]. The images from the experiment chip are from an internal dataset.

eight modules arranged in a cyclical process. For image acquisition, the system utilizes SOTA research microscope setups and low-cost 3D-printed microscope systems in the first EAP module. Second, the real-time image processing is executed on incoming images, generating single-cell instance segmentation predictions. The generated data and metadata are saved and managed in an instance of the third module's OMERO DataBase (DB). This instance is also used to manage ground truth data generated with the cell simulator module CellSium and the ObiWan-Microbi semi-annotation module as the fourth and fifth modules. Sixth, the real-time data analysis module relies on this data to provide feedback via a dashboard and detect events. Based on these, the real-time experiment planner, as the seventh module, schedules the experiment continuously and sends the next steps to the microscope control module, the eighth module, which forwards these instructions back to the image acquisition module. The modules are described individually in the upcoming sections.

3.1 Microscope Control

µManager. Automatic control of the microscope is essential for experiment automation. To make our EAP as independent as possible from microscope manufacturers and thus enable easy transfer to new laboratories and microscopes, µManager [11] is used. µManager is an open-source software package that controls microscopes and associated hardware components such as cameras, stages and shutters. It provides a powerful, flexible and cost-effective solution for automated microscopy. In this work, the implementation is done in Python with the help of Pymmcore(-Plus)³ with a Nikon T1-based setup.

Autofocusing. One specific challenge we address here is the autofocusing of the microscope. We treat autofocusing as a regression problem, where a simple Multi-Layer Perceptron (MLP) is used to predict the relationship between the extracted input features from microscopy images and the continuous target variable, which is the distance to the optimal focus frame (among all z-stacks). After predicting the focus offset and direction, our system employs a closed-loop control mechanism to communicate the predicted adjustments to the microscope control. The focus is then iteratively adjusted until the optimal focus is reached.

3.2 Image Acquisition

This module handles image acquisition primarily in two different ways:

1. The most common process is using real research microscopes for the experiments and generating high-quality images. This is still by far the most used approach, particularly due to the ability of such microscopes to provide direct, high-resolution and high-fidelity images of biological samples.
2. The low-cost alternative to such expensive tools are 3D-printed microscopes, which are emerging as cost-effective and accessible tools,

³ <https://github.com/micro-manager/pymmcore>

especially in educational settings and low-resource environments [8]. However, they generally do not match the resolution and functionality of high-end commercial microscopes.

The acquired image data and metadata are then collected and saved according to standardized protocols in a OMERO DB. Standardization offers the possibility of a uniform mask for querying different information for all modules (including future ones). Furthermore, the data can be distributed, stored and accessed worldwide.

3.3 Real-Time Image Processing

The image data acquired in the previous step (see Section 3.2) is processed as shown in Fig. 1. On the one hand, the region of interest, that is the growth chamber, is extracted by removing any microfluidic structures from the images. On the other hand, the content of the chamber is segmented using a suitable method. In this work, we focus on SOTA DL segmentation methods (see Section 2), which are either task-specific, domain-specific or foundation models and therefore allow us to address various segmentation tasks effectively. We also investigate the data processing speed of these methods. This is important because the classification of the events and, therefore, the decision of the experiment planner (see Section 3.8) is highly based on the segmentation results.

3.4 OMERO Database

As mentioned in Section 2, we use OMERO to manage not only the images and the associated metadata in a centralized and standardized manner but also the results of downstream analyses such as chamber detection and extraction, segmentation and cell analysis. All modules of the EAP (see Fig. 1) can retrieve information via a standardized interface. In addition, this makes it possible for the human expert to easily and comprehensibly document his experiments, including access to the post-processing and -analysis results.

3.5 ObiWan-Microbi

For intra- and inter-cell analysis to be possible, the best feasible extraction of objects through segmentation (see Section 3.3) is required. One challenge in our context is the large number of densely packed cells that need to be segmented. To date, there are no labeled datasets that accurately represent a comparable use case, which would facilitate transfer learning or the training of supervised segmentation methods. Since manual labeling alone would be too long and too inefficient, the semi-automated annotation tool ObiWan-Microbi is used in this work. This tool allows the prediction and correction of labels and subsequent retraining of the used DL segmentation models. An example of a dataset created with this is [51], which will be used to evaluate the segmentation methods in Section 4.

3.6 CellSium

However, even the creation of labels using semi-automated methods such as ObiWan-Microbi (see Section 3.5) costs a lot of human time and therefore money, especially in the first iteration because the segmentation methods still provide right-angled pre-segmentations. An alternative here is to have an initial basis for the segmentation methods by using automatically generated images with associated labels, e.g., from simulations. The simulator CellSium is used in our work. CellSium enables the generation of microbe images in different growth stages and also in the density and frequency required in our context. Even if these images cannot represent the full diversity of real images, combining data augmentation methods can lead to first stable results as shown in [45], where only slight adjustments have to be made in ObiWan-Microbi.

3.7 Real-Time Data Analysis

3.7.1 Dashboard

Once the microbes have been segmented, single-cell data such as average cell size and growth rate are computed and saved. This data is visualized

and, most importantly, leveraged by the human expert to navigate through the experiment. For this purpose, a customized dashboard is being developed. The dashboard provides real-time insights into ongoing experiments such as cell count, growth rate and average cell size per chamber. The dashboard integrates various functionalities to facilitate the monitoring and analysis of the experiment. Due to its modular architecture, which facilitates the seamless integration of new features and functionalities without disrupting the existing codebase, our dashboard is highly extendable and can be tailored for other use cases.

3.7.2 Event Detection

The data and metadata gathered from the real-time data analysis and image processing enable us to detect different events in hundreds of parallel experiments and resolve their temporal evolution. In our case, we have two classes of events. On the one hand, technical events that are related to the devices themselves, e.g., loss of focus or chamber defects. On the other hand, we have biological events that display the behavior of microbes, such as growth rate or cell death. The detection is performed based on rules provided by the domain expert.

3.8 Real-Time Experiment Planner

A central part of the EAP pipeline is the intelligent experiment planner. The next n chambers to be explored are determined based on the last chamber recorded, including the resulting data analysis. The determination is made according to the defined experiment objectives of the human domain expert.

4 Experiments

In this section, preliminary experiments and results of our approach, particularly for real-time image segmentation, are presented and discussed. Four segmentation algorithms are compared on an Ubuntu 22.04-based workstation with an Intel Core i9-13900 Central Processing Unit (CPU), a RTX3090

Graphics Processing Unit (GPU) and a 64 GB Random-Access Memory (RAM). This system was chosen as low-performance because the hardware components represent an affordable system for users interested in such use cases. The measured inference time can be considered realistic for a lower boundary. An improved hardware configuration can achieve an additional performance boost here. We define 100 ms as the maximal limit for the real-time inference time. This is because initial tests of the microscope control program have shown that it is perfectly sufficient for the EAP4EMSIG, including autofocusing.

4.1 Dataset, Metrics and Implementation

The benchmark dataset [51] is used to evaluate the methods. The dataset contains images of *Corynebacterium glutamicum* microbes and represents a typical experiment in cell culture. The dataset includes video sequences of the development of the microbes with 800 images each and consists of ground truth instance segmentation mask and tracking paths. For the context of this work, we use all 5×800 images purely to evaluate the segmentation performances.

To evaluate the segmentation accuracy, the metrics AP, including AP@0.50 and AP@0.75 and PQ, comprising Segmentation Quality (SQ) and Recognition Quality (RQ), are calculated for all four methods mentioned in Section 2 (see Table 1) using their respective official implementations.

Since the AP-based metric requires the confidence score for calculation, evaluating this metric on Omnipose was impossible. Omnipose does not directly return uncertainties for predicted masks and no official instructions on how to extract these are known until the publication of the work.

4.2 Real-Time Image Processing: Segmentation

The evaluation results of the four methods are shown in Table 1. In addition, an example image from the dataset (see Fig. 2a) and the respective segmentation results (see Fig. 2b to Fig. 2e) are displayed for a medium population density with approx. 400 microbes (see Fig. 2).

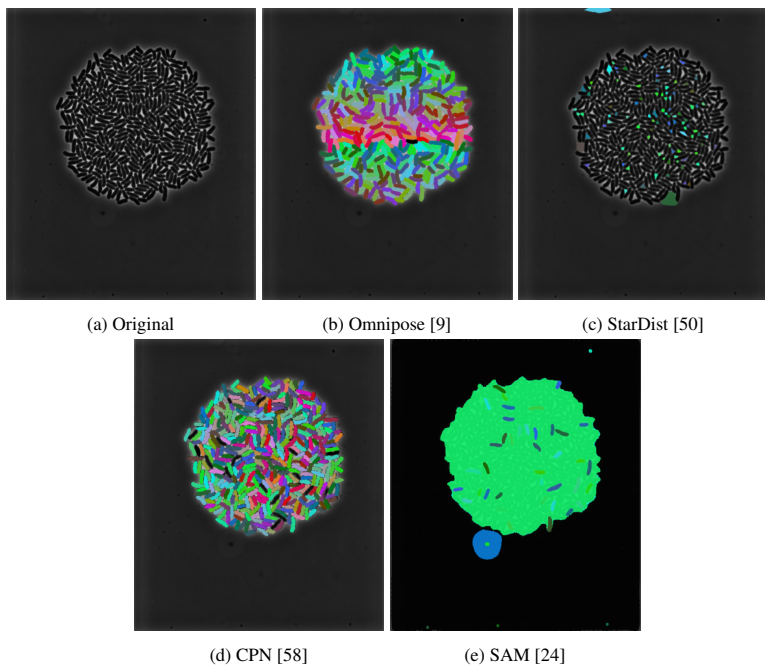


Figure 2: Comparison of zero-shot instance segmentation predictions for [51]. The original image is shown in Fig. 2a and the predictions are shown in Fig. 2b to Fig. 2e.

From the results in Table 1, Omnipose is the best model concerning the scores PQ, SQ and RQ. However, CPN with a PQ score difference of 0.0761, i.e., domain-specific model, is not that far away and is still 86 ms faster than Omnipose. In detail, CPN has a slightly lower RQ score of 0.0526, but the difference in PQ score is primarily due to the notably worse SQ. The SQ score can be seen by directly comparing Fig. 2b and Fig. 2d. While Omnipose segmented the objects cleanly, including at the edges, CPN struggled. Additionally, some parts are often no longer properly recognized in the curved microbes towards the end.

Nevertheless, CPN’s performance is quite remarkable because in CPN’s training datasets, there were no such long, rod-shaped objects or similar microbial colonies in contrast to the task domain model Omnipose. As a domain model, it is also remarkable that CPN recognizes the object instances with a similarly

Method Metric	Omnipose [9]	StarDist [50]	CPN [58]	SAM-H [24]
AP↑	-	0.0000	0.6232	0.0347
AP@0.5↑	-	0.0000	0.9551	0.0476
AP@0.75↑	-	0.0000	0.8170	0.0470
PQ↑	0.9336	0.3629	0.8575	0.0626
PQ-SQ↑	0.9395	0.7287	0.8779	0.8416
PQ-RQ↑	0.9935	0.4093	0.9763	0.0736
∅Inf. [ms]↓	271	7686	185	1994

Table 1: Average Precision (AP) results, Panoptic Quality (PQ) results comprising Segmentation Quality (SQ) score and Recognition Quality (RQ) score as well as inference times (Inf.) evaluated on the benchmark dataset [51].⁴

good RQ score (only 0.0172 difference). Both the number of objects and the difficult boundaries between the objects are not new for CPN and also occur, for example, in *NeurIPS 22 Cell Segmentation Competition*⁵ dataset as one of the pre-training datasets. However, in combination with the microbes, this is a noteworthy generalization achievement.

The second domain model StarDist, on the other hand, with a PQ score of 0.3629 and an AP score of 0, has not yielded sufficient results and is clearly worse than CPN. The vision foundation model Segment Anything, the fourth model, is also not convincing with a PQ score of 0.0626. However, it is worth noting that the SQ score is only slightly lower than that of CPN, indicating that the objects recognized as True Positive (TP) were segmented well. However, upon examining RQ, it appears that the number of TP is likely very low. Segment Anything’s problem can also be seen in Fig. 2e. There, almost the entire cluster of microbes is predicted as one object. Although there are many objects in dense clusters in the dataset SA-1B [24] on which the model was trained and also with a comparable number, it is quite possible that this transferred knowledge cannot be applied to the shape, which in turn raises doubts about the “Any” segmentation.

⁵ <https://neurips22-cellseg.grand-challenge.org/>

⁴ When calculating the metric, falsely detected backgrounds were not removed and evaluated during the AP calculation as false positives. The models were used according to the basic configurations for fair comparison. The values in bold are the best across all methods, provided there were results. To ensure a fair comparison, we define inference time as the duration from inputting

5 Conclusion

This paper presents the EAP4EMSIG - a novel pipeline for experiment automation for thousands of microbe colonies on microfluidic chips. For this purpose, the methodological concept of each of the eight pipeline modules was introduced, explained and distinguished from existing alternatives. Preliminary development steps of the pipeline were presented, particularly for the real-time image segmentation module. To this end, four SOTA methods were compared and evaluated quantitatively and qualitatively in the paper. CPN and Omnipose proved to be particularly powerful. Omnipose, trained task-specifically for bacteria segmentation, is 86 ms slower at inference than CPN but has a slightly better recognition quality and a noticeably higher segmentation quality. However, because CPN was not trained explicitly for bacterial segmentation, but on very diverse biomedical cells such as blood cells or nuclei of different cell types, among others, future work would investigate retraining different methods to get the best model for real-time segmentation in the EAP4EMSIG. Future work will also investigate increasing segmentation speed to achieve the minimum 100 ms required for our task, such as converting models to special inference formats like TensorRT⁵ or transforming the trained models to a lower precision (e.g. FP16, Int8).

Even though only initial results for the real-time image processing module were shown in this work, the other modules are also being developed. So far, the pipeline as a whole has not yet been tested, but the modules themselves are already in advanced development and, in some cases, ready for use, such as CellSium or ObiWan-Microbi. The next steps are to combine the modules and test the EAP4EMSIG as a whole.

the image to receiving the model's prediction as an instance mask with confidence scores. This includes any post-processing needed by certain methods, such as converting predicted contours to a pixel-wise mask for the EAP4EMSIG pipeline. The inference time is measured with all models using FP32 precision.

⁵ <https://github.com/NVIDIA/TensorRT>

Acknowledgments

This work was supported by the President's Initiative and Networking Funds of the Helmholtz Association of German Research Centres [Grant EMSIG ZT-I-PF-04-44]. The Helmholtz Association funds this project under the "Helmholtz Imaging Platform", the authors N. Friederich, A. J. Yamachui Sitcheu and R. Mikut under the program "Natural, Artificial and Cognitive Information Processing (NACIP)", the authors N. Friederich and A. J. Yamachui Sitcheu through the graduate school "Helmholtz Information & Data Science School for Health (HIDSS4Health)" and the author Johannes Seiffarth through the graduate school "Helmholtz School for Data Science in Life, Earth and Energy (HDS-LEE)".

The authors have accepted responsibility for the entire content of this manuscript and approved its submission. We describe here the individual contributions of N. Friederich (NF), A. J. Yamachui Sitcheu (AJYS), A. Nassal (AN), M. Pesch (MP), E. Yildiz (EY), M. Beichter (MB), L. Scholtes (LS), B. Akbaba (BA), T. Lautenschlager (TL), O. Neumann (ON), D. Kohlheyer (DK), H. Scharr (HS), J. Seiffarth (JS), K. Nöh (KN), R. Mikut (RM): Conceptualization: NF, AJYS, JS, RM; Methodology: NF, AJYS, EY, JS, DK, HS, KN, RM; Software: NF, AN, MB; Investigation: NF, AJYS, JS; Resources: JS, KN; Writing – Original Draft: NF, AJYS, MP, MB, ON, EY, JS; Writing – Review & Editing: NF, AJYS, AN, MP, EY, MB, LS, BA, TL, ON, NK, HS, JS, KN, RM; Supervision: DK, HS, KN, RM; Project administration: DK, HS, KN, RM; Funding Acquisition: DK, HS, EY, KN, RM.

References

- [1] C. Allan, J.-M. Burel, J. Moore, C. Blackburn, M. Linkert, S. Loynton, D. MacDonald, W. J. Moore, C. Neves, A. Patterson, et al. OMERO: flexible, model-driven data management for experimental biology. *Nature Methods*, 9(3):245–253, 2012.

- [2] U. Bethe, Z. D. Pana, C. Drosten, H. Goossens, F. König, A. Marchant, G. Molenberghs, M. Posch, P. van Damme, and O. A. Cornely. Innovative approaches for vaccine trials as a key component of pandemic preparedness - a white paper. *Infection*, 2024.
- [3] K. M. Carbone, R. B. Luftig, and M. Buckley. *Microbial Triggers of Chronic Human Illness*. Am Soc Microbiol, 2005.
- [4] A. E. Carpenter, B. A. Cimini, and K. W. Eliceiri. Smart microscopes of the future. *Nature Methods*, 20(7):962–964, 2023.
- [5] A. Casadevall. Microbes and Climate Change - Science, People & Impacts. 2022.
- [6] L. Chiron, M. Le Bec, C. Cordier, S. Pouzet, D. Milunov, A. Banderas, J.-M. Di Meglio, B. Sorre, and P. Hersen. CyberSco. Py an open-source software for event-based, conditional microscopy. *Scientific Reports*, 12(1):11579, 2022.
- [7] A. Christovich and X. M. Luo. Gut microbiota, leaky gut, and autoimmune diseases. *Frontiers in Immunology*, 13:946248, 2022.
- [8] J. T. Collins, J. Knapper, J. Stirling, J. Mduda, C. Mkindi, V. Mayagaya, G. A. Mwakajinga, P. T. Nyakyi, V. L. Sanga, D. Carbery, L. White, S. Dale, Z. J. Lim, J. J. Baumberg, P. Cicuta, S. McDermott, B. Vodenicharski, and R. Bowman. Robotic microscopy for everyone: the OpenFlexure microscope. *Biomed. Opt. Express*, 11(5):2447–2460, 2020.
- [9] K. J. Cutler, C. Stringer, T. W. Lo, L. Rappez, N. Stroustrup, S. Brook Peterson, P. A. Wiggins, and J. D. Mougous. Omnipose: a high-precision morphology-independent solution for bacterial cell segmentation. *Nature Methods*, 19(11):1438–1448, 2022.
- [10] P. Dettinger, T. Frank, M. Etzrodt, N. Ahmed, A. Reimann, C. Trenzinger, D. Loeffler, K. D. Kokkaliaris, T. Schroeder, and S. Tay. Automated microfluidic system for dynamic stimulation and tracking of single cells. *Analytical Chemistry*, 90(18):10695–10700, 2018.

- [11] A. D. Edelstein, M. A. Tsuchida, N. Amodaj, H. Pinkard, R. D. Vale, and N. Stuurman. Advanced methods of microscope control using μ Manager software. *Journal of Biological Methods*, 1(2), 2014.
- [12] A. Esteva, K. Chou, S. Yeung, N. Naik, A. Madani, A. Mottaghi, Y. Liu, E. Topol, J. Dean, and R. Socher. Deep learning-enabled medical computer vision. *npj Digital Medicine*, 4(1):5, 2021.
- [13] H. Fakhuruldeen, G. Pizzuto, J. Glowacki, and A. I. Cooper. Archemist: Autonomous robotic chemistry system architecture. In *2022 International Conference on Robotics and Automation (ICRA)*, pages 6013–6019. IEEE, 2022.
- [14] M. S. Fasihi and W. B. Mikhael. Overview of current biomedical image segmentation methods. In *2016 International Conference on Computational Science and Computational Intelligence (CSCI)*, pages 803–808, 2016.
- [15] A. Fischer-Nielsen, Z. Fu, T. Su, and A. Wąsowski. The forgotten case of the dependency bugs: on the example of the robot operating system. In *Proceedings of the ACM/IEEE 42nd International Conference on Software Engineering: Software Engineering in Practice*, pages 21–30, 2020.
- [16] Z. R. Fox, S. Fletcher, A. Fraisse, C. Aditya, S. Sosa-Carrillo, J. Petit, S. Gilles, F. Bertaux, J. Ruess, and G. Batt. Enabling reactive microscopy with MicroMator. *Nature Communications*, 13(1):2199, 2022.
- [17] N. Friederich, A. J. Yamachui Sitcheu, O. Neumann, S. Eroglu-Kayıkçı, R. Prizak, L. Hilbert, and R. Mikut. AI-based automated active learning for discovery of hidden dynamic processes: A use case in light microscopy. In *Proceedings-33. Workshop Computational Intelligence: Berlin, 23.-24. November 2023*, volume 23, page 31. KIT Scientific Publishing, 2023.
- [18] M. Ganesan, R. Mani, S. Sai, G. Kasivelu, M. K. Awasthi, R. Rajagopal, N. I. Wan Azelee, P. K. Selvi, S. W. Chang, and B. Ravindran. Bioremediation by oil degrading marine bacteria: An overview of

supplements and pathways in key processes. *Chemosphere*, 303(Pt 1):134956, 2022.

- [19] P. Godau and L. Maier-Hein. Task Fingerprinting for Meta Learning in Biomedical Image Analysis. In *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2021*, pages 436–446. Springer, 2021.
- [20] F. He and L. Zhang. Design of indoor security robot based on robot operating system. *Journal of Computer and Communications*, 11(5):93–107, 2023.
- [21] H. Jeckel and K. Drescher. Advances and opportunities in image analysis of bacterial cells and communities. *FEMS Microbiology Reviews*, 45, 2020.
- [22] W. Ji, J. Li, Q. Bi, T. Liu, W. Li, and L. Cheng. Segment Anything Is Not Always Perfect: An Investigation of SAM on Different Real-world Applications. *Machine Intelligence Research*, 21(4):617–630, Aug 2024.
- [23] A. Kirillov, K. He, R. Girshick, C. Rother, and P. Dollár. Panoptic segmentation. In *Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition*, pages 9404–9413, 2019.
- [24] A. Kirillov, E. Mintun, N. Ravi, H. Mao, C. Rolland, L. Gustafson, T. Xiao, S. Whitehead, A. C. Berg, W.-Y. Lo, P. Dollár, and R. Girshick. Segment Anything. *arXiv:2304.02643*, 2023.
- [25] G. Lancini and A. L. Demain. Bacterial pharmaceutical products. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors, *The Prokaryotes*, pages 257–280. Springer Berlin Heidelberg, Berlin, Heidelberg, 2013.
- [26] A. Lehmussola, P. Ruusuvuori, J. Selinummi, H. Huttunen, and O. Yli-Harja. Computational framework for simulating fluorescence microscope images with cell populations. *IEEE Transactions on Medical Imaging*, 26(7):1010–1016, 2007.

- [27] M. Li, A. Fang, X. Yu, K. Zhang, Z. He, C. Wang, Y. Peng, F. Xiao, T. Yang, W. Zhang, X. Zheng, Q. Zhong, X. Liu, and Q. Yan. Microbially-driven sulfur cycling microbial communities in different mangrove sediments. *Chemosphere*, 273:128597, 2021.
- [28] T.-Y. Lin, M. Maire, S. Belongie, J. Hays, P. Perona, D. Ramanan, P. Dollár, and C. L. Zitnick. Microsoft COCO: Common Objects in Context. In *Computer Vision—ECCV 2014: 13th European Conference, Zurich, Switzerland, September 6-12, 2014, Proceedings, Part V 13*, pages 740–755. Springer, 2014.
- [29] B.-N. Liu, X.-T. Liu, Z.-H. Liang, and J.-H. Wang. Gut microbiota in obesity. *World Journal of Gastroenterology*, 27(25):3837–3850, 2021.
- [30] K. J. Locey and J. T. Lennon. Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 113(21):5970–5975, 2016.
- [31] A. Lou, S. Guan, and M. Loew. CFPNet-M: A Light-Weight Encoder-Decoder Based Network for Multimodal Biomedical Image Real-Time Segmentation. *Computers in Biology and Medicine*, 154:106579, 2023.
- [32] S. Macenski, T. Foote, B. Gerkey, C. Lalancette, and W. Woodall. Robot Operating System 2: Design, architecture, and uses in the wild. *Science Robotics*, 7(66):eabm6074, 2022.
- [33] D. Mahecic, W. L. Stepp, C. Zhang, J. Griffié, M. Weigert, and S. Manley. Event-driven acquisition for content-enriched microscopy. *Nature Methods*, 19(10):1262–1267, 2022.
- [34] B. Miles and P. L. Lee. Achieving Reproducibility and Closed-Loop Automation in Biological Experimentation with an IoT-Enabled Lab of the Future. *SLAS TECHNOLOGY: Translating Life Sciences Innovation*, 23(5):432–439, 2018.
- [35] M. Molina-Moreno, M. P. Schilling, M. Reischl, and R. Mikut. Automated Style-Aware Selection of Annotated Pre-Training Databases in Biomedical Imaging. In *2023 IEEE 20th International Symposium on Biomedical Imaging (ISBI)*, pages 1–5, 2023.

- [36] J. M. Nduko and S. Taguchi. Microbial production of biodegradable lactate-based polymers and oligomeric building blocks from renewable and waste resources. *Frontiers in Bioengineering and Biotechnology*, 8:618077, 2020.
- [37] J. P. Neto, A. Mota, G. Lopes, B. J. Coelho, J. Frazão, A. T. Moura, B. Oliveira, B. Sieira, J. Fernandes, E. Fortunato, R. Martins, R. Igreja, P. V. Baptista, and H. Águas. Open-source tool for real-time and automated analysis of droplet-based microfluidic. *Lab Chip*, 23:3238–3244, 2023.
- [38] K. Oliphant and E. Allen-Vercoe. Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome*, 7(1):91, 2019.
- [39] E. Pedone, I. De Cesare, C. G. Zamora-Chimal, D. Haener, L. Postiglione, A. La Regina, B. Shannon, N. J. Savery, C. S. Grierson, M. Di Bernardo, et al. Cheetah: a computational toolkit for cybergenetic control. *ACS Synthetic Biology*, 10(5):979–989, 2021.
- [40] H. Pinkard, N. Stuurman, I. E. Ivanov, N. M. Anthony, W. Ouyang, B. Li, B. Yang, M. A. Tsuchida, B. Chhun, G. Zhang, et al. Pycro-Manager: open-source software for customized and reproducible microscope control. *Nature Methods*, 18(3):226–228, 2021.
- [41] H. Pinkard and L. Waller. Microscopes are coming for your job. *Nature Methods*, 19(10):1175–1176, 2022.
- [42] D. M. S. Pinto, M. A. Phillips, N. J. Hall, J. Mateos-Langerak, D. Stoychev, T. S. Pinto, M. J. Booth, I. Davis, and I. M. Dobbie. Python-Microscope – a new open-source Python library for the control of microscopes. *Journal of Cell Science*, 134, 2021.
- [43] F. Rahmanian, J. Flowers, D. Guevarra, M. Richter, M. Fichtner, P. Donnelly, J. M. Gregoire, and H. S. Stein. Enabling modular autonomous feedback-loops in materials science through hierarchical experimental laboratory automation and orchestration. *Advanced Materials Interfaces*, 9(8):2101987, 2022.

- [44] O. Ronneberger, P. Fischer, and T. Brox. U-Net: Convolutional Networks for Biomedical Image Segmentation. In *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015*, pages 234–241. Springer, 2015.
- [45] C. C. Sachs, K. Ruzaeva, J. Seiffarth, W. Wiechert, B. Berkels, and K. Nöh. CellSium: versatile cell simulator for microcolony ground truth generation. *Bioinformatics Advances*, 2(1):vbac053, 2022.
- [46] S. Sanchez and A. L. Demain. Useful microbial enzymes—an introduction. In *Biotechnology of Microbial Enzymes*, pages 1–11. Elsevier, 2017.
- [47] T. Scherr, J. Seiffarth, B. Wollenhaupt, O. Neumann, M. P. Schilling, D. Kohlheyer, H. Scharr, K. Nöh, and R. Mikut. microbeSEG: A deep learning software tool with OMERO data management for efficient and accurate cell segmentation. *Plos one*, 17(11):e0277601, 2022.
- [48] M. P. Schilling, S. Schmelzer, L. Klinger, and M. Reischl. KaIDA: a modular tool for assisting image annotation in deep learning. *Journal of Integrative Bioinformatics*, 19(4):20220018, 2022.
- [49] C. Schmidt, J. Hanne, J. Moore, C. Meesters, E. Ferrando-May, S. Weidtkamp-Peters, et al. Research data management for bioimaging: the 2021 NFDI4BIOIMAGE community survey. *F1000Research*, 11, 2022.
- [50] U. Schmidt, M. Weigert, C. Broaddus, and G. Myers. Cell Detection with Star-Convex Polygons. In *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2018*, pages 265–273. Springer, 2018.
- [51] J. Seiffarth, L. Blöbaum, K. Löffler, T. Scherr, A. Grünberger, H. Scharr, R. Mikut, and K. Nöh. Data for - Tracking one in a million: Performance of automated tracking on a large-scale microbial data set. <https://doi.org/10.5281/zenodo.7260137>, 10 2022.
- [52] J. Seiffarth, L. Blöbaum, R. Paul, N. Friederich, A. J. Yamachui Sitcheu, R. Mikut, H. Scharr, A. Grünberger, and K. Nöh. Tracking one-in-a-million: Large-scale benchmark for microbial single-cell tracking

with experiment-aware robustness metrics. In *European Conference on Computer Vision*. Springer, 2024.

- [53] J. Seiffarth, T. Scherr, B. Wollenhaupt, O. Neumann, H. Scharr, D. Kohlheyer, R. Mikut, and K. Nöh. ObiWan-Microbi: OMERO-based integrated workflow for annotating microbes in the cloud. *SoftwareX*, 26:101638, 2024.
- [54] R. Sender, S. Fuchs, and R. Milo. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology*, 14(8):e1002533, 2016.
- [55] A. Y. Sitcheu, N. Friederich, S. Baeuerle, O. Neumann, M. Reischl, and R. Mikut. MLOps for Scarce Image Data: A Use Case in Microscopic Image Analysis. In *Proceedings-33. Workshop Computational Intelligence: Berlin, 23.-24. November 2023*, volume 23, page 169. KIT Scientific Publishing, 2023.
- [56] D. Svoboda and V. Ulman. MitoGen: A Framework for Generating 3D Synthetic Time-Lapse Sequences of Cell Populations in Fluorescence Microscopy. *IEEE Transactions on Medical Imaging*, 36:310–321, 2017.
- [57] D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel, and D. A. Zuberer. *Principles and applications of soil microbiology*. Pearson, 2005.
- [58] E. Upschulte, S. Harmeling, K. Amunts, and T. Dickscheid. Contour proposal networks for biomedical instance segmentation. *Medical Image Analysis*, 77:102371, 2022.
- [59] M. D. Wilkinson, M. Dumontier, I. J. Aalbersberg, G. Appleton, M. Axton, A. Baak, N. Blomberg, J.-W. Boiten, L. B. da Silva Santos, P. E. Bourne, et al. The FAIR Guiding Principles for scientific data management and stewardship. *Scientific Data*, 3(1):1–9, 2016.
- [60] B. Xiao, H. Wu, W. Xu, X. Dai, H. Hu, Y. Lu, M. Zeng, C. Liu, and L. Yuan. Florence-2: Advancing a unified representation for a variety of vision tasks. In *Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition*, pages 4818–4829, 2024.