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ObiWan-Microbi: OMERO-based integrated workflow for annotating microbes in the cloud

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ABSTRACT

Automated microscopy techniques combined with high-throughput microfluidic cultivation systems provide unique insights into living microorganisms, enabling hundreds of experiments to be performed in parallel. Such setups generate large data sets with rich cellular features that need to be extracted to arrive at quantitative insights. The sheer amount of recorded time-lapse images requires reliable automated processing. While recent advances in deep learning methods have enabled automated processing, these methods rely on large-scale and precisely annotated data sets, often not available for new organisms or custom imaging modalities.

To overcome the annotated data bottleneck, particularly in the microbial domain, we present the open-source *ObiWan-Microbi* platform providing a fast workflow for large-scale annotation of up to hundred thousands of segmentation and tracking annotations in time-lapse imaging data. *ObiWan-Microbi* focuses on easy-to-use semi-automated annotation in the browser eliminating the need for local installation or accelerator hardware, encourages FAIR data management using *OMERO*, and provides convenient collaborative cloud deployment to simplify the creation and long-term development of large-scale annotated data sets. The public availability of such benchmark data sets has the potential to improve data-driven methods, increase comparability among them, and is an essential step towards reliable automated image processing in microbial live-cell microscopy.

Current code version	v0.1.1
Permanent link to code/repository used for this code version	https://github.com/ElsevierSoftwareX/SOFTX-D-23-00468
Permanent link to Reproducible Capsule	https://github.com/hip-satomi/ObiWan-Microbi/releases/tag/v0.1.1
Legal Code License	MIT
Code versioning system used	git
Software code languages, tools, and services used	Python, Typescript, Angular, Ionic, Docker
Compilation requirements, operating environments & dependencies	Linux (tested: Ubuntu-22.04) or Windows (WSL, Ubuntu 22.04 LTS)1J4E, Docker
If available Link to developer documentation/manual	https://github.com/hip-satomi/ObiWan-Microbi/
Support email for questions	j.seiffarth@fz-juelich.de

1. Motivation and significance

Automated live-cell imaging has become an exceptionally valuable tool for life scientists to investigate the spatio-temporal development of cell populations at the single-cell level [1–3]. Recent advances in microfluidic systems unlocked the simultaneous cultivation of microbial cultures in hundreds of picoliter bioreactors on a single lab-on-chip device [4,5]. In combination with either phase-contrast or fluorescence imaging, microfluidic live-cell imaging (MLCI) allows performing experiments under various continuous cultivation conditions

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[6–8], while observing intracellular processes, such as the production of specific chemical products [9,10] or the consequences of cellular stress responses [11]. Thus, MLCI turned into a versatile research tool with unique capabilities to elucidate the enigmatic emergence of phenotypic heterogeneity of microbes [12], to shed light into the interactions within microbial communities [13,14], and promises to become increasingly relevant for modern bioprocess development [8,15].

In MLCI experiments, high amounts of isogenic cells are repeatedly imaged over multiple generations to be representative of the complexity and variety of microbial behavior and, eventually, to generate conclusive biological insights. In a typical experiment featuring exponentially growing microbes, 300 growth chambers are imaged every five minutes for one day (generating ≈ 800 GB), resulting in millions of cell instances. Hence, extracting relevant biological information such as cell sizes, the development of fluorescence in single cells, or growth rates from the acquired time-lapse image sequences requires robust and automated image processing workflows. Two crucial steps in these processing workflows are the identification of individual cells (*cell instance segmentation*) and their association through time (*cell tracking*).

Several advanced deep learning (DL) models have been developed for cell segmentation [16–19], cell tracking [20,21], or simultaneous cell tracking and segmentation [22] to establish fully automated image processing. The common key to success in these approaches is the availability of extensive high-quality (i.e., pixel-perfect) ground truth (GT) annotation for effectively training and validating DL models [23,24]. In the biomedical domain, the collection of such annotated data sets has been established and diverse images and image sequences have been annotated by domain experts and in crowdsourcing efforts [25], with the manual workload reaching up to thousands of work hours [26]. As a result, DL models, trained with these annotated data sets, are now empowering comprehensive image analysis pipelines [21,26–30]. A particular example of successful cross-fertilization is the Cell Tracking Challenge (CTC), which provides public benchmarks for biomedical segmentation and tracking methods tailored to eukaryotic cells that push the further development of methods in this field [31]. In contrast, and with only sparse exceptions [32], there is a notable lack of open large-scale MLCI benchmark data sets, holding back similar breakthroughs in the microbial domain [33]. Here, benchmark data capturing the unique challenges of MLCI images – the formation of dense cell colonies, with often simple shapes but frequent cell division, which makes it difficult to identify and track individual cells – are markedly different from existing CTC data sets and, therefore, essential for fine-tuning DL methods and models to microbes and validating automated analysis methods [23].

Clearly, creating benchmark data resembling the domain-specific MLCI challenges requires an efficient annotation tool for segmentation and tracking, scaling to thousands of cell instances in a single image and thousands of cell tracks per time-lapse sequence. In contrast, in the CTC 2D data sets the maximal number of cell instances in a single image is 651 and the maximal number of divisions in a single sequence is 569. Given these characteristics, establishing a toolset for efficient, at best semi-automated, annotation of microbial image data is key to progress. Specifically, annotation tools need to be simple to operate, require minimal installation effort, and not be overloaded with functionality to ease the entry for and adoption by domain experts. Furthermore, FAIR data management, i.e., meeting findability, accessibility, interoperability and reusability principles, is essential for long-term and continuous development of public benchmark data sets [34].

To address the needs, we developed the *ObiWan-Microbi* software platform that incorporates these features into a single and easy-to-use workflow (cf. Fig. 1). For *ObiWan-Microbi*, we developed the browser-based annotation software *AnnUI* as key component for large-scale segmentation and tracking annotation in the browser without any local installation. It utilizes the existing *OMERO* server to support FAIR data management, and integrates state-of-the-art DL segmentation methods for semi-automated annotation executed on a remote server using the developed *SegServe* software.

The web app *AnnUI* connects to an *OMERO* data storage and provides an efficient user interface for segmentation and tracking annotation of time-lapse sequences, scaling to hundreds of thousands of annotations and supporting convenient touch or pen devices for high-precision annotation. For scenarios where automated segmentation already yields decent segmentation predictions, we developed *SegServe*, a REST API providing an extendable model zoo for remote DL segmentation execution that eliminates hardware barriers for the end-user, and enables an accelerated semi-automated annotation workflow. *ObiWan-Microbi* combines the existing *OMERO* data management platform, with our newly developed *AnnUI* and *SegServe* tools into a microservice architecture, is open-source, and comes with detailed video tutorials. Together, *ObiWan-Microbi* provides an efficient workflow for creating large-scale annotated benchmark data sets to push forward reproducible and comparable segmentation and tracking development in the MLCI domain.

2. Software description

The primary objective of *ObiWan-Microbi* is to provide an efficient and user-friendly annotation workflow for MLCI experiments. In order to achieve this, we make three underlying software design decisions: First, we adopted existing DL segmentation methods and developed the service *SegServe* that makes them remotely executable using a REST API. Second, we developed the browser-based user interface *AnnUI*, optimized for efficient large-scale time-lapse annotation and semi-automated annotation, eliminating the need for local software installation or special hardware resources on the user side. Third, we package all software components as containers to make them easy to maintain and deploy them as microservices alongside the *OMERO* platform.

2.1. Remote deep learning segmentation execution

In practice, semi-automated annotation using existing DL segmentation methods is often difficult for end-users due to extensive hardware and software requirements. Providing accelerator hardware such as GPUs to all users and managing software versions is very cost and time intensive. Thus, we developed *SegServe*, a REST API designed to execute DL segmentation methods with GPU acceleration remotely on a central compute server. *SegServe* utilizes *FastAPI* to provide the REST backend, but it also comes with an automatically generated user interface. The instance segmentation method is defined by its git URL, version number, and parameters. Together with the image, this information is sent via a REST request, the method is installed and executed on the remote server, and the resulting instance segmentation is returned in JavaScript Object Notation (JSON) format. The DL segmentation methods are bundled in git repositories and installed automatically with all their software dependencies using *MLFlow* (https://mlflow.org/) dependency management and *anaconda* environments (https://www.anaconda.com/). The on-demand installation guarantees reproducible code execution for different model or software versions and secures adaptability to future DL segmentation methods. Moreover, the software installations are cached and reused for all subsequent segmentation requests. We integrated three off-the-shelf DL segmentation methods for microbial image analysis, namely *Cellpose* [18], *Omnipose* [19] and *HTC* [17] trained on simulated image data [35]. In addition, *Yolov5* is available for general bounding-box object detection [36].

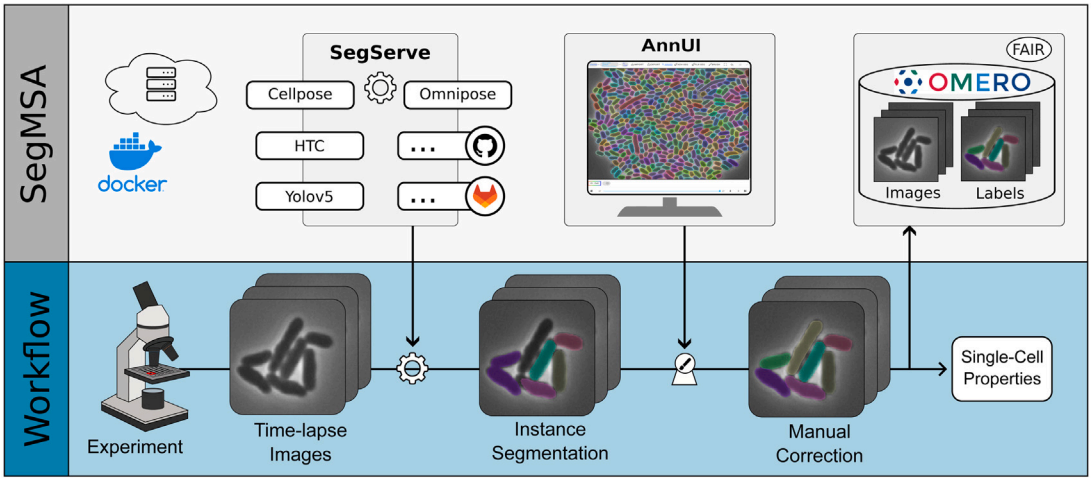

Fig. 1. *ObiWan-Microbi* platform overview. Fully integrated end-to-end workflow for semi-automated image annotation of MLCI time-lapse microscopy in the browser. *ObiWan-Microbi* consists of *SegServe*, *AnnUI*, and *OMERO* jointly distributed in the microservice architecture *SegMSA*.

2.2. Efficient browser-based segmentation and tracking annotation in time-lapse images

To unlock the efficiency of semi-automated annotation workflows, we developed the user-friendly web app *AnnUI*. The application is based on the Angular and Ionic frameworks (https://angular.io/, https://ionic.io/) and is implemented using Typescript (https://www.typescriptlang.org/). *AnnUI* connects to the *OMERO* data backend and allows for navigating image data. A comprehensive feature set for both manual and semi-automated segmentation annotation of time-lapse sequences leverages the capabilities of the *SegServe* service. Therefore, using *AnnUI* across large and interdisciplinary research teams is simplified as no local software installation and maintenance is needed.

AnnUI provides an optimized tool set for convenient and fast manual annotation, including a brush tool for drawing and correcting segmentation annotations, a multi-selection tool to remove annotations in a rectangular area, and multi-label annotations for complex imaging data (Fig. 2). The data model records every annotation action separately, providing undo or redo functionality. Therefore, mistakes during the annotation process can be corrected without losing progress. In addition, keeping track of all annotation actions allows quantifying the amount of manual annotation work needed for every single image in a time-lapse sequence. To prevent annotation loss due to network connectivity problems, the data model is automatically synchronized with the *OMERO* backend using *RxJS* events (https://rxjs.dev/).

For high-precision annotation, *AnnUI* supports touch inputs and can be used with professional pen devices or consumer tablets. For semi-automated annotation, three established DL segmentation and one bounding box detection model are provided for prediction, followed by a correction using the manual annotation tools. To integrate custom DL segmentation approaches or fine-tuned hyperparameters, *AnnUI* allows deriving new model configurations for the *SegServe* API.

Based on the cell instance annotation, *AnnUI* facilitates the creation of tracks for individual cells, explicitly focusing on minimizing the number of mouse clicks and improving the quality of the manual annotations. Tracking cells in MLCI is very challenging due to frequent division, dense cell colonies, and the uniform visual appearance. Thus, it is often necessary to incorporate the temporal context of a cell and its neighborhood to make the correct manual tracking links. We integrated a tracking workflow that emphasizes this temporal context. Upon selecting a cell instance, the subsequent image in the time-lapse is automatically presented to enable the selection of the next cell instance in the track. This sequential procedure is continued until the end of the cell cycle, i.e., from cell appearance to division or disappearance, and gives the user the temporal context of the cell development (Fig. 2 FG). The cell track is completed with a cell division or disappearance annotation. Furthermore, already annotated cell tracks are visualized using a gray arrow for the movement in the previous frame and blue or red arrows for the movement towards the next frame for dividing and non-dividing cells, respectively (Fig. 2 F).

To annotate all cell tracks, we incorporated a quick finder method highlighting the next cell instance that still needs to be tracked. Furthermore, any erroneous tracks or links can easily be corrected using the undo/redo functionality or the cutting tool, which allows users to draw a line to remove all intersecting tracking links (Fig. 2).

2.3. Microservice architecture

To facilitate FAIR data principles, *ObiWan-Microbi* uses the *OMERO* service for image data handling and annotation storage, including user and access management. Thus, software compatible with *OMERO*, such as *Fiji* [37] or *Napari* [38] can directly use the annotations for further analysis, for example, to extract single-cell properties.

ObiWan-Microbi combines *AnnUI*, *SegServe*, and *OMERO* into a Docker-based microservice architecture (Fig. 3). The modularization into containers and the definition of their network interactivity allow for a convenient installation procedure of the full platform with only three commands in the command line. During development, errors in the setup are detected using continuous integration (CI) pipelines that automatically build and test the individual containers. Furthermore, the microservice architecture allows the integration of custom containers, adapting the platform to existing infrastructure, or deploying it in the cloud.

All in all, *ObiWan-Microbi* establishes a workflow tailored to MLCI data and designed for efficiency, usability and scalability to support large-scale annotation efforts. Its extensible microservice architecture and the remote execution of new DL models keeps installation and future maintenance efforts minimal.

3. Illustrative examples

We present the functionality of the *ObiWan-Microbi* annotation with two examples. First, we demonstrate automated DL segmentation on microbial images to underline the importance of having a model zoo to choose from when performing semi-automated annotation. Second, we showcase large-scale data sets that have already been created using the platform.

During semi-automated annotation, the workload for manual correction highly depends on the performance of existing DL segmentation models. Fig. 4 shows the segmentation results displayed in the

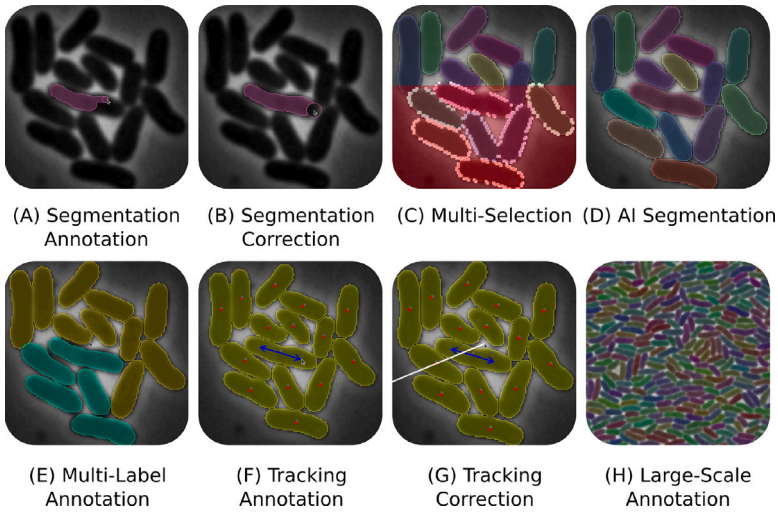

Fig. 2. Overview of *AnnUI* functionality: Manual segmentation annotation (A); correction using the brush tool (B); multi-selection for artifact removal (C); DL segmentation proposals for semi-automated annotation and manual error correction (D); multi-label annotation (E); manual tracking annotation by drawing linking edges between segmentation annotations in consecutive images (F); the removal of tracking links using a track cutting tool that allows drawing a cut (white line) and removes all intersecting track links (G); visualization of a large amount of detected cells with random color for instance separation (H).

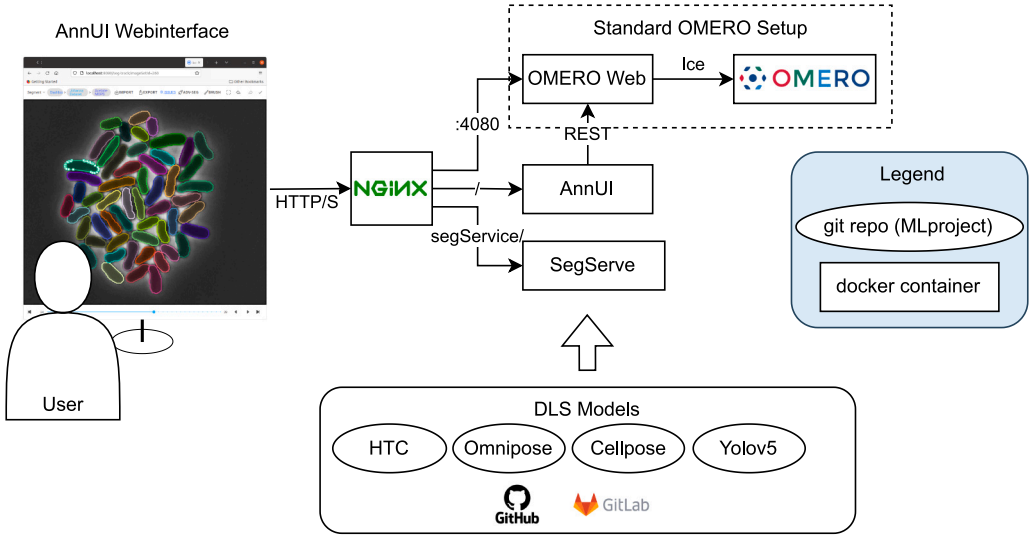

Fig. 3. The *ObiWan-Microbi* microservice architecture: An *NGINX* reverse proxy organizes the HTTP traffic between the microservices. The *SegServe* service downloads and installs DL segmentation (DLS) models on demand from git repositories, for example, hosted on GitHub or GitLab.

AnnUI interface for exemplary phase-contrast images of three established microbial organisms, namely *E. coli*, *C. glutamicum*, and *B. subtilis*. We observe that *Omnipose* performs the best pre-annotation for a follow-up manual correction, while *HTC* and *Cellpose* require higher manual annotation efforts. Using *ObiWan-Microbi*, users are empowered to qualitatively assess the best working DL segmentation approach for their specific use cases, concentrating annotation efforts on erroneous segmentation only. We have achieved annotation speeds of more than 200 cells per minute using the semi-automated segmentation workflow.

The shortcomings of existing DL segmentation methods can be reduced by iteratively retraining models on newly annotated data, following the human-in-the-loop principle. Due to its annotation capabilities and interoperability, *ObiWan-Microbi* is already used within the *microbeSEG* software [39] that complements the workflow by generating training data and retraining of DL segmentation methods on cropped images. Annotated segmentation data[1] and trained DL segmentation models[2] are made publicly available.

Furthermore, the fast semi-automated workflow of *ObiWan-Microbi*, handling thousands of annotations per image, has already been used for large-scale and complete MLCI time-lapse annotation. A first data set for *C. glutamicum* experiments including 1.4 million cell instances and more than $29k$ cell tracks in five time-lapse sequences is also made publicly available[3] and serves as a strong baseline for developing new automated methods and validating their suitability for automated application.

[1] https://doi.org/10.5281/zenodo.6497714
[2] https://doi.org/10.5281/zenodo.7221151
[3] https://doi.org/10.5281/zenodo.7260136

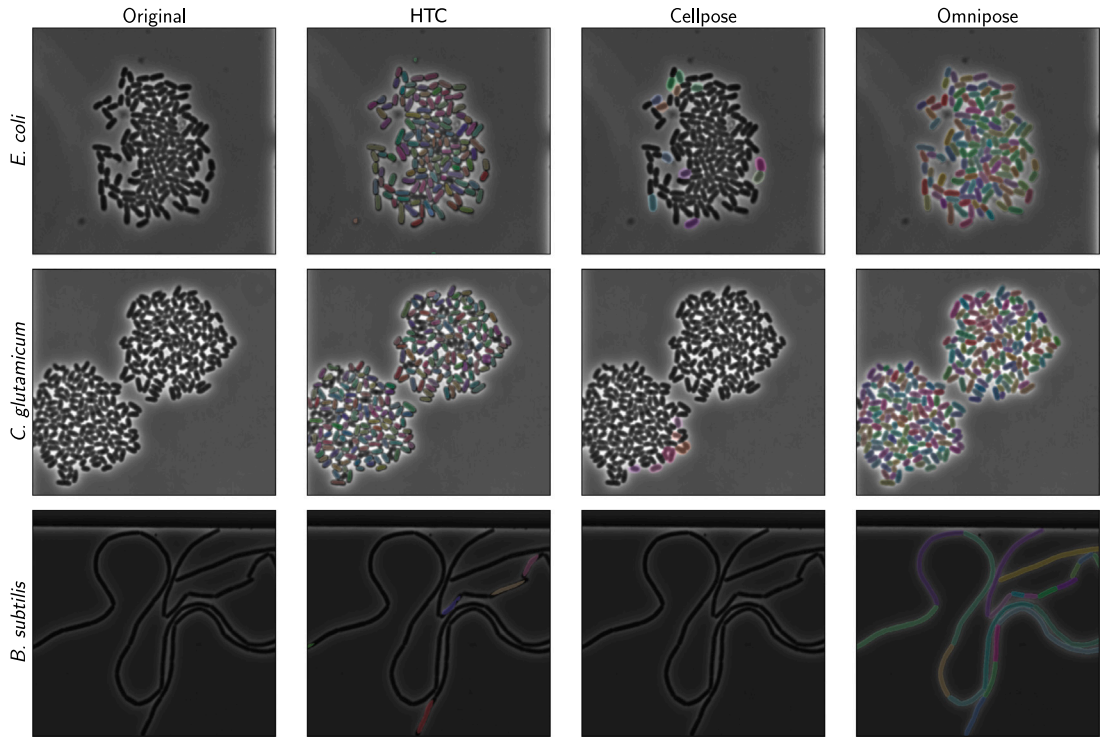

Fig. 4. Application of DL segmentation methods *HTC*, *Cellpose* and *Omnipose* inside the *AnnUI* web interface to microfluidic live-cell image data containing *E. coli*, *C. glutamicum* and *B. subtilis* cells.

4. Impact

ObiWan-Microbi provides the complete annotation workflow of segmentation and tracking tailored for time-lapse MLCI. Its support of FAIR data management, state-of-the-art DL segmentation methods, and collaborative annotation makes it especially useful for interdisciplinary image processing and large-scale annotation efforts. Using and integrating existing best-practices and methods ensures interoperability with existing downstream processing (e.g. *Fiji*). The easy and reproducible usage of DL segmentation methods removes time-consuming manual setups, allowing to focus on fast and precise annotation.

While initially developed and optimized for MLCI annotation, the data-integrated workflow of *ObiWan-Microbi* has the potential to be used in other imaging domains, including plant images, biomedical cell images, material sciences, or satellite images. Due to its expendable DL segmentation architecture, even tailored deep learning solutions for these domains can be incorporated, and the multi-label annotation aids in performing complex image annotation. These domains also benefit from FAIR data usage and collaborative annotation opportunities for precise image analysis and large-scale benchmark data set generation. However, given the particular application field (microbes), currently, the workflow is based on closed-polygon annotations, limiting its ability to annotate complex shapes, holes or disconnected components.

5. Conclusion

ObiWan-Microbi is a collaborative annotation platform providing an efficient workflow for creating high-precision annotations for segmenting and tracking in microbial time-lapse imaging. Its focus on efficient large-scale annotation, encouragement of FAIR data management, expandable DL segmentation methods, and minimal user hardware requirements simplifies annotation efforts in interdisciplinary research domains. Based on growing annotated data sets, combined with human-in-the-loop workflows, *ObiWan-Microbi* provides the foundation for iteratively improving data and methods and pushing publicly available benchmark data sets for the training and validating novel methods in the MLCI domain. As we have recently seen in the biomedical field, this development is essential for establishing reliable and fully automated image analysis pipelines and unlocking the full potential of time-lapse MLCI for investigating biological phenomena.

CRediT authorship contribution statement

Johannes Seiffarth: Conceptualization, Methodology, Software, Validation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Tim Scherr:** Software, Writing – review & editing. **Bastian Wollenhaupt:** Data curation, Software. **Oliver Neumann:** Software. **Hanno Scharr:** Funding acquisition, Writing – review & editing. **Dietrich Kohlheyer:** Funding acquisition, Resources. **Ralf Mikut:** Funding acquisition, Writing – review & editing. **Katharina Nöh:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

We have shared the links to used data used and our code in the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.softx.2024.101638.

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