microbeSEG: A deep learning software tool with OMERO data management for efficient and accurate cell segmentation

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Abstract

In biotechnology, cell growth is one of the most important properties for the characterization and optimization of microbial cultures. Novel live-cell imaging methods are leading to an ever better understanding of cell cultures and their development. The key for analyzing acquired data is accurate and automated cell segmentation at the single-cell level. Therefore, we present microbeSEG, a user-friendly Python-based cell segmentation tool with graphical user interface and OMERO data management. microbeSEG utilizes a state-of-the-art deep learning-based segmentation method and can be used for instance segmentation of a wide range of cell morphologies and imaging techniques, e.g., phase contrast or fluorescence microscopy. The main focus of microbeSEG is a comprehensible, easy, efficient, and complete workflow from creation of training data to final application of the trained segmentation model. We demonstrate that accurate cell segmentation results can be obtained within 45 minutes of user time. Utilizing public segmentation datasets or pre-labeling further accelerates the microbeSEG workflow. This opens the door for accurate and efficient data analysis of microbial cultures.

Introduction

Cell-to-cell heterogeneity induced by intrinsic biological factors and extrinsic environmental fluctuations highly affects microbial cell growth and productivity of microbes [1–6]. State-of-the-art microfluidic lab-on-chip systems with highly precise environmental control and time-lapse imaging enable to investigate causes and consequences of such heterogeneity at the single-cell level [7–9]. Interesting growth parameters include number of cells over time, or size distributions. To extract such quantities from the acquired image sequences, all cells in all images need to be individually segmented; a process that needs to be highly automated and reliable due to the high numbers of cells (up to several tens of thousands of cells per experiment). However, virtually error-free segmentation of acquired time-lapse images remains a challenge, as contrast and signal-to-noise ratio are typically low due to limited lighting conditions to avoid phototoxic stress.

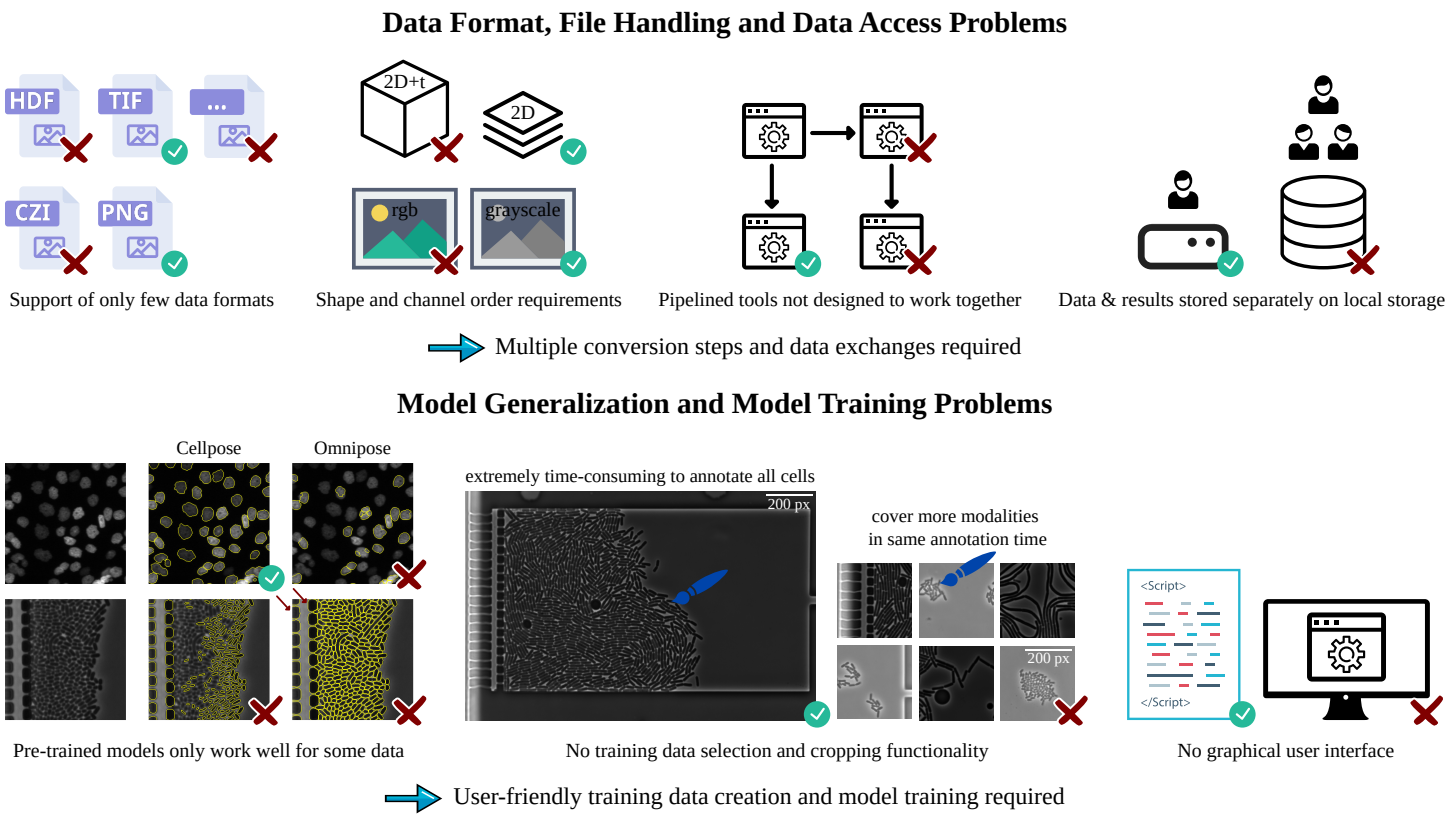


Fig 1. Typical barriers end users face when using segmentation software. Cell segmentation methods are typically designed for specific applications or for segmentation challenges and therefore lack a data management system with a versatile data importer. This results in additional file format and image shape conversion steps. Furthermore, often multiple tools need to be combined to cover the whole workflow from training data creation to the application of trained models. Again, additional conversion steps may be needed since the tools are not designed to work together. The model generalization and model training problems show that generally it is (not yet) possible to do without own annotated training data and that interactive cropping functionalities are needed for dense growing organisms. Shown segmentations were made with Cellpose [12] and Omnipose [13] (fluorescence images inverted for Omnipose and phase contrast images inverted for Cellpose).

Cell segmentation tools are therefore often highly specialized to the specific imaging conditions and need to be adapted when acquisition settings or experimental parameters change, or to conform with other cell morphologies or image modalities. When the tools are based on traditional image processing methods, as e.g. ChipSeg [10], some adaptations can be achieved by manually adjusting parameters. Unfortunately, this often requires some expert knowledge on the underlying method and in some cases even code adaptations. In contrast, deep learning-based methods are not tuned manually and much more versatile, but need to be (re-)trained on annotated data (see Fig 1). Due to this versatility deep learning methods nowadays dominate cell segmentation challenges covering multiple object morphologies and imaging techniques, e.g., the 2018 Data Science Bowl [11].

Training a deep learning-based method involves a pipeline consisting of multiple steps usually covered by a variety of sequentially applied tools or programming steps. A typical workflow starts with training data creation, data handling and loading, model training, model evaluation – these steps may be iterated until the model is suitably accurate – and finally the application of trained models to experiment data. From a user perspective, integrating all needed steps in a single, highly automated toolbox is desirable. In particular, the data management, the training data creation, and the training of

the deep learning models need to be user-friendly and time efficient, e.g., by using pre-labeling with subsequent manual corrections and full automation of routine steps. Thus far, deep learning approaches for the segmentation of biological data either lack a smooth training data creation and retraining workflow, or a data management system like OMERO [14]. Recent examples are Misic for the high-throughput cell segmentation of complex bacterial communities [15], and Omnipose for robust segmentation of bacteria and other elongated cell shapes [13]. Fig 1 summarizes some typical barriers for end users when using cell segmentation software.

In this paper, we present microbeSEG, an open-source deep learning-based segmentation tool which uses the freely available OMERO [14] for data management. microbeSEG is a tool tailored to instance segmentation of various cell morphologies and imaging techniques. In contrast to other solutions, microbeSEG covers the whole processing pipeline needed for image analysis: users can easily create training datasets, annotate cells with the jointly developed annotation toolkit ObiWan-Microbi [16], and train and apply deep learning models – without any conversion steps or programming. Due to the use of OMERO and its versatile data importer, supporting over 150 image formats, data management is straightforward and user-friendly. microbeSEG provides powerful deep learning-based cell segmentation in an easy-to-use tool and is publicly available at https://github.com/hip-satomi/microbeSEG. In the remainder of this work, the microbeSEG functionalities and workflow are introduced, the workflow efficiency is evaluated and the key features of available cell segmentation software are compared.

Materials and methods

Fig 2 provides an overview of the microbeSEG functionalities and its needed components OMERO for data management and ObiWan-Microbi, a jointly developed tool for manual annotation. The key features are the use of OMERO and the completeness of the workflow which includes time-efficient interactive training data crop selection. microbeSEG facilitates deep learning for biologists since no data format conversion steps are needed for data annotation, model training and inference. Such steps are needed when tools need to be combined that are not designed to work together and no data management system providing file format standards are used. The functionalities and the components of microbeSEG are described in this section.

OMERO

OMERO is an open-source software platform for access and use of a wide range of biological data and provides a unified interface for images, matrices and tables [14]. Over 150 image formats can be imported. Data are organized in projects and datasets. With the OMERO.web client, data can easily be accessed and viewed in the browser.

ObiWan-Microbi

ObiWan-Microbi is an open-source microservice platform for segmentation ground truth annotation in the cloud [16]. The Angular and Ionic based web app connects to an OMERO data backend and has been developed jointly with microbeSEG. For pre-labeling, Omnipose [13] and Cellpose [12] can be used.

Segmentation methods

In microbeSEG, two deep learning-based instance segmentation methods are implemented: (i) a simple semantic-based method with multi-class output, i.e., background, cell

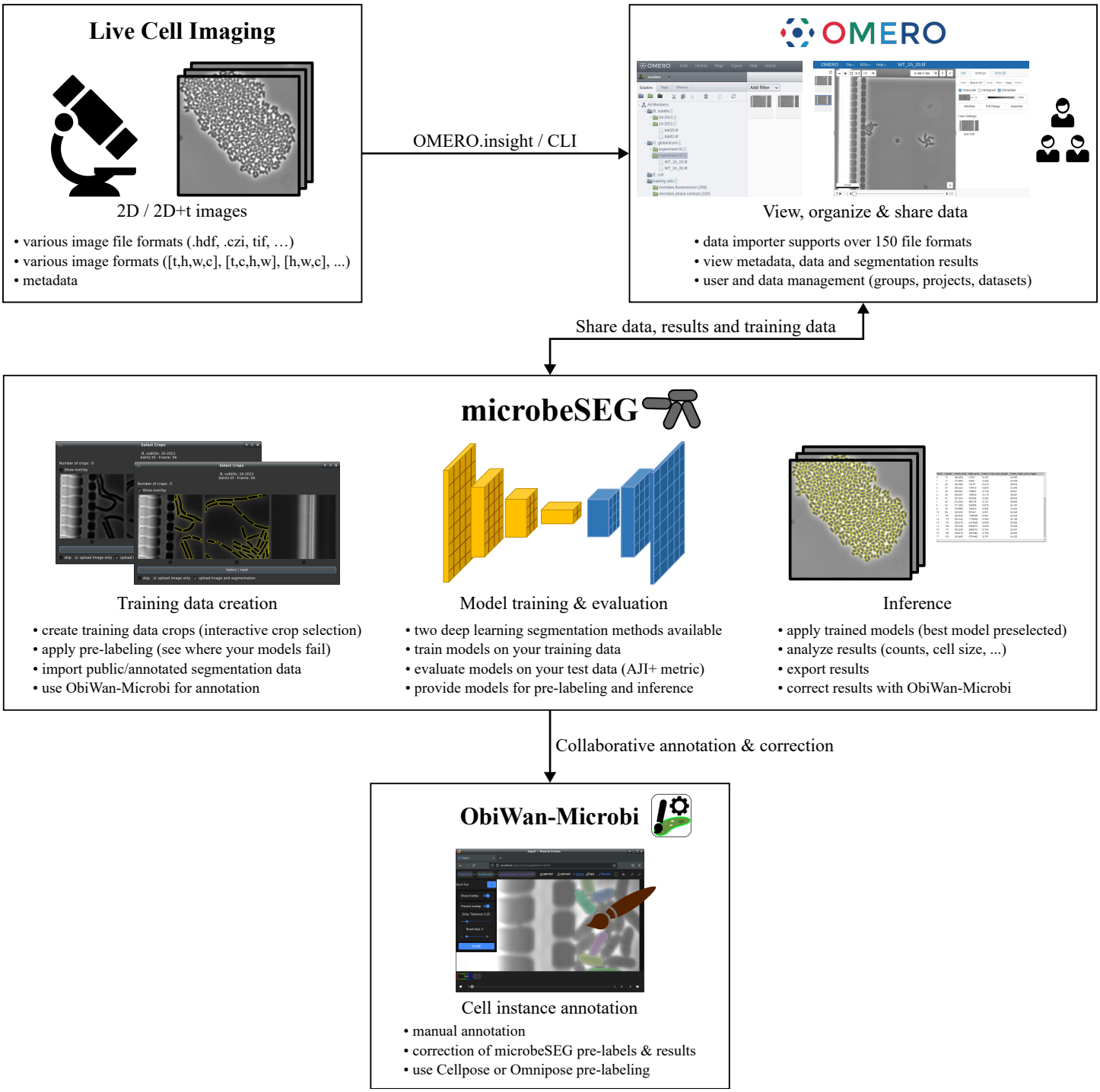
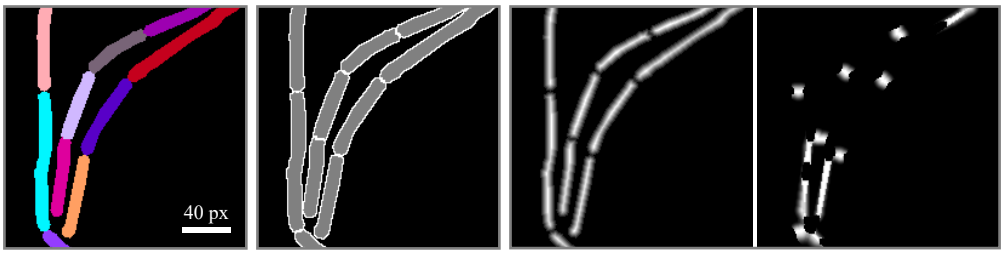


Fig 2. microbeSEG workflow. OMERO is used for data management and provides a versatile data importer and standardizes file handling [14]. Data can be viewed in the browser with the OMERO.web client. microbeSEG offers training data creation, training, evaluation, and inference functionalities. The jointly developed toolkit ObiWan-Microbi is used for manual annotation and result correction [16].



(a) Ground truth (b) Boundary method (c) Distance method

Fig 3. Training data representations of the two microbeSEG methods. From the ground truth (a – instances: color-coded) the boundary representation (b – cell interior: gray, cell boundary: white) and the distance representations (c – distance to background, inverse distance to neighbors) can be computed. A deep learning model is trained to predict either the boundary representation or the distance representations and the single instances are recovered in the post-processing.

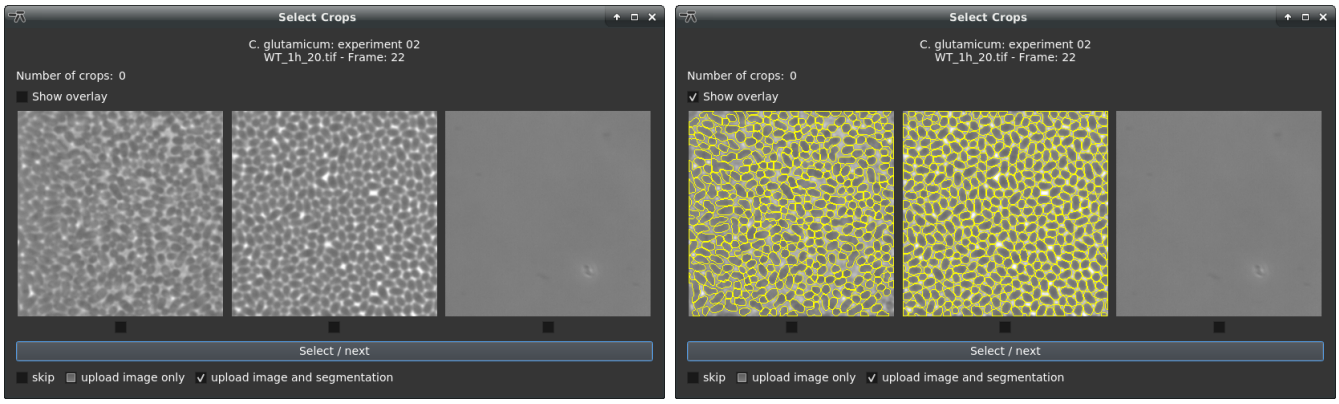
interior and cell boundary (see Fig 3b), and (ii) a distance transform-based method (see Fig 3c) [17,18]. The latter method (ii) has proven its applicability for phase contrast, bright field and fluorescence images for diverse cell morphologies in the Cell Tracking Challenge (http://celltrackingchallenge.net/) [17,19] where the method currently holds nine top-three rankings as participants KIT-GE (2) and KIT-GE (3). The simple boundary method is easy to interpret and serves as a baseline. Both methods are based on the U-Net convolutional neural network [20], with the distance method using two decoders, one for each predicted output. In the seeded watershed-transform-based post-processing, touching instances are split using the cell boundary prediction or the neighbor distance prediction. The distance method post-processing has two parameters, a threshold for adjusting the cell size and a threshold adjusting the seed extraction.

Training data creation

microbeSEG training sets are managed as OMERO datasets. When adding a new training set to OMERO by using the graphical user interface of microbeSEG, a crop size needs to be selected. This is required since annotating whole images or frames is tedious and time-consuming, e.g., for frames with microbial colonies of hundreds or thousands of cells. More efficient is to annotate smaller crops showing less cells (see Fig 1). This enables to cover more image and cell features in the same annotation time and results in a more diverse training set. Therefore, crop proposals of selected files can be viewed and added to the training set (Fig 4a). Up to three crops – depending on the image and crop size – randomly extracted from non-overlapping image regions are shown per frame and can interactively be selected and uploaded to OMERO. When trained models are already available, pre-labeling enables to identify regions where the segmentation fails and improvements are needed (Fig 4b). In addition, this approach reduces the annotation time for correctly or partially correctly segmented cells. Selected crops are automatically assigned to a training, validation or test subset. However, there is also an option for explicit assignment. The crops can then be viewed and annotated using ObiWan-Microbi. Furthermore, annotated datasets, e.g., publicly available training data, can be imported. With the OMERO.web client, the training data and the annotations can be viewed as well.

Training

For training on a selected annotated training set, the segmentation method, the batch size, the optimizer and how many models should be trained need to be specified. Adam [21]



(a) Without overlay (b) With overlay

Fig 4. Crop creation interface. Automatically proposed crops can be selected and uploaded to OMERO (a). The crop proposals are extracted randomly from different non-overlapping image regions (the left crop originates from the left image region and the right crop from the right). For the pre-labeling, it is possible to upload only the image or the image with its prediction (b). The image only upload is helpful when the pre-label predictions require too much manual corrections.

and Ranger [22] are available as optimizer, each with its own pre-defined settings, i.e., start learning rate, minimum learning rate, and learning rate scheduler (reduce learning rate on validation loss plateau). The default settings are: distance method, Ranger optimizer, training of five models, and a batch size of four. Usually, the default settings are sufficient but the batch size may be reduced by the user if memory availability is limited. Flipping, rotation, scaling, contrast, blur and noise augmentations are applied during training. The maximum number of training epochs is set automatically depending on the dataset and crop size. In addition, a stopping criterion depending on the maximum number of training epochs and validation loss improvement is applied. For the boundary method, a weighted sum of cross entropy loss and channel-wise Dice loss is used. The distance method is trained with a smooth L_1 loss.

Evaluation

Trained models can be evaluated on the automatically split test set. Thereby, appropriate parameters are set for the post-processing of the distance method, i.e., the cell size adjustment and the seed extraction threshold. As evaluation measure, a slightly modified version of the aggregated Jaccard index AJI [23] is used. The modification AJI+ prevents overpenalization by using a one-to-one mapping instead of a one-to-many mapping for the predicted objects [24, 25]. The AJI+ score ranges from 0 to 1, with 1 indicating perfect segmentation. The mean AJI+ score over the single test images and its standard deviation of each evaluated model are saved in a csv file.

Inference and result export

For inference, the best evaluated model with its parameter set is selected automatically. However, the user can also select the model manually. If a not yet evaluated model is selected, the default thresholds are used for the distance method which may result in (slightly) too large or too small cells for this method. The segmentation results can be attached to the corresponding OMERO image as polygon region of interests, which enables to store images and results together, and can be viewed with the OMERO.web client. In addition, the cell counts, mean cell area, mean cell minor and major axis length, and the total cell area are determined. The result export includes: the original

Table 1. microbeSEG accuracy in dependence of annotation time. All methods are evaluated on 12 *B. subtilis* and 12 *E. coli* test images. For each microbeSEG setting, the median aggregated Jaccard index AJI+ out of five trained models is shown. The times include the crop selection times.

Manual annotation time	N_{crops}	N_{cells}	Method	AJI+ (*B. subtilis*)	AJI+ (*E. coli*)	AJI+ (total)
30 min†	13	309	boundary (Adam)	0.484	0.653	0.568
			distance (Ranger)	0.585	0.729	0.657
30 min† + 15 min‡	18	732	boundary (Adam)	0.505	0.687	0.596
			distance (Ranger)	0.614	0.731	0.673
pre-trained	-	-	Omnipose	0.478	0.519	0.498

† *Same training dataset.*

‡ *With pre-labeling using a distance method model trained on the dataset annotated in the first 30 minutes.*

image (.tif), intensity-coded instance segmentation masks (.tif), a cell outlines image (.tif), the original image overlaid with the cell outlines (.tif), and analysis results (.csv).

Implementation, installation and dependencies

microbeSEG is implemented in Python [26] and uses PyTorch as deep learning framework [26, 27]. The graphical user interface is build with PyQt [28]. The software and the source code are available under the MIT license in our code repository. A requirements file and a step-by-step guide are provided in the code repository for easy installation and usage. Access to an OMERO server is required for running microbeSEG. For testing purposes, a demo server account can be requested from the OME team in Dundee: http://qa.openmicroscopy.org.uk/registry/demo_account/. Furthermore, an OMERO server can be set up during the ObiWan-Microbi installation which is available at https://github.com/hip-satomi/ObiWan-Microbi.

microbeSEG dataset

Microbe data shown in this paper were acquired with an fully automated time-lapse phase contrast microscope setup using a 100x oil immersion objective. Cultivation took place inside a special microfluidic cultivation device [29]. The assembled microbe training and test sets including 2930 cell in total are available online at https://doi.org/10.5281/zenodo.6497715 (see next section).

Results

Workflow efficiency

Table 1 shows the microbeSEG segmentation accuracy in terms of the aggregated Jaccard index AJI+ in dependence of the annotation time on a test set consisting of two microbe types, i.e., of 721 *B. subtilis* and 1168 *E. coli* cells. The 24 test images of size $320\,\text{px} \times 320\,\text{px}$, 12 for each species, were annotated by three experts, who cross-checked their annotations. The microbeSEG user selected and annotated crops from different experiment data than the experts have used. The distance method parameters have been adjusted automatically on the test set of the microbeSEG user.

Switching from completely manual annotation to the correction of pre-labeled image crops after the initial annotation phase results in a higher number of cells annotated per time. Therefore, a model trained on the dataset annotated in the first 30 minutes has been used. The additional training data increase the scores for both cell types further, especially for the simpler boundary method which requires larger training datasets. The

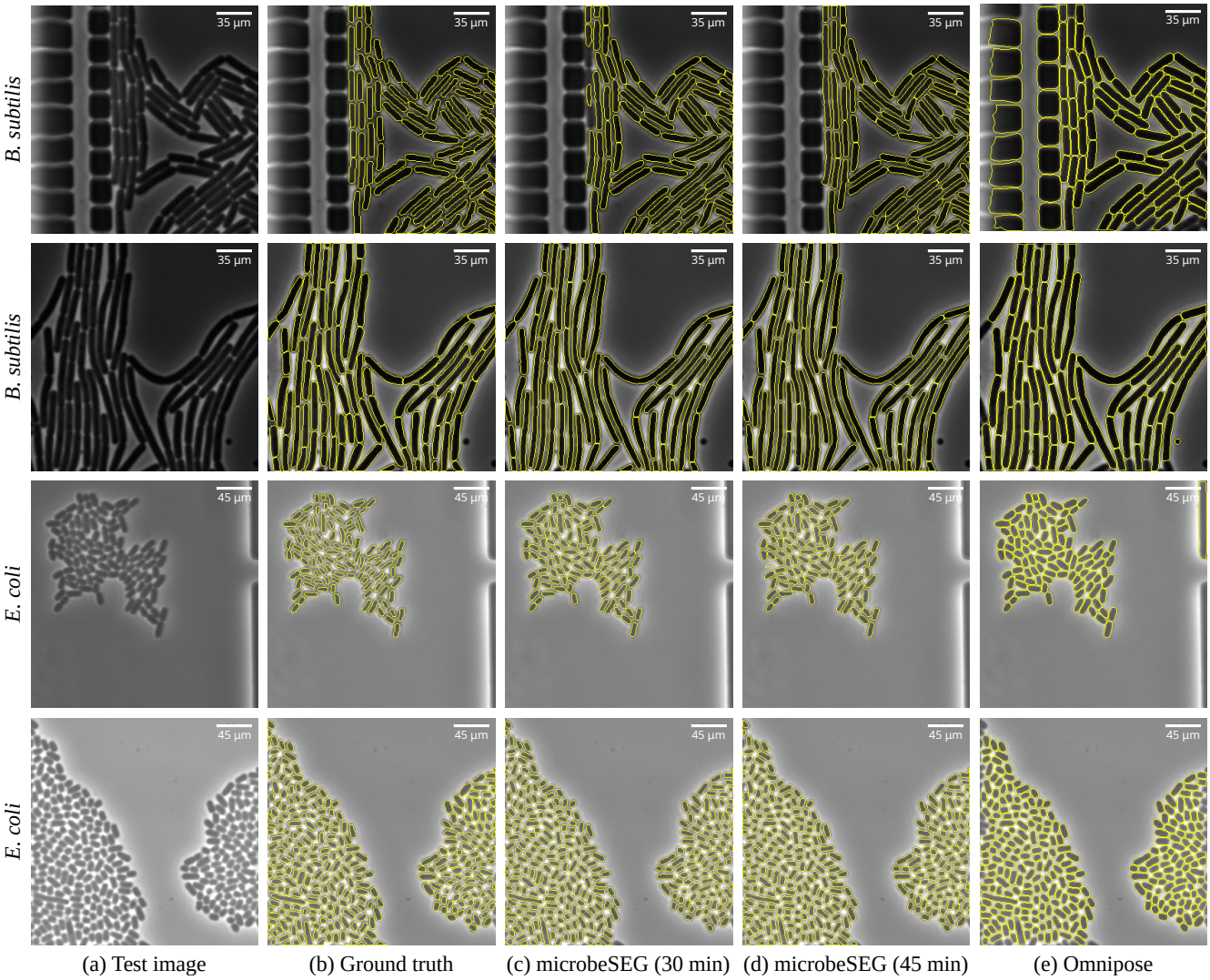
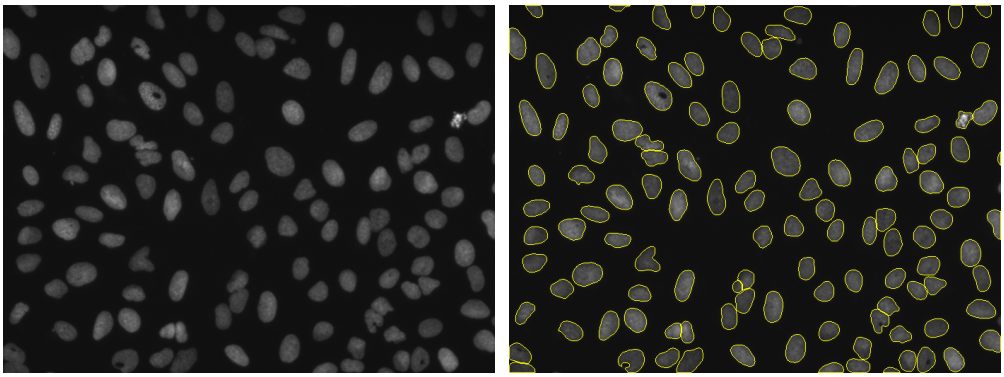


Fig 5. Exemplary images and segmentation overlays for *B. subtilis* and *E.coli*. Shown are the results of the median distance method microbeSEG model reported in Table 1.

default microbeSEG settings – distance method and Ranger optimizer – yield better segmentations than the boundary method. Fig 5 shows exemplary segmentations for the default settings which indicate that reasonable segmentation results for the two microbe types can be obtained with 30 minutes annotation time, even without utilizing public datasets, pre-training or pre-labeling. For comparison, Omnipose [13], a deep learning method pre-trained on a large database of bacterial images with 27 000 annotated cells, yields convincing segmentations of cells, but without re-training suffers from the detection of background structures.

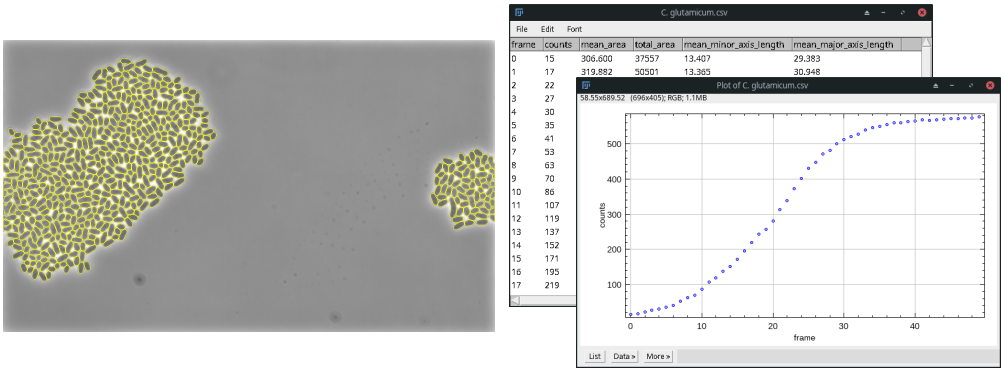
Data import

The import of annotated datasets helps to further decrease manual interaction time, or even make the need for manual annotations or corrections obsolete in some cases where very similar training data is available. Fig 6 shows qualitative segmentation results of U2OS cells [30] with a microbeSEG model trained on HeLa cell data from the Cell



(a) Image (b) Image-segmentation overlay

Fig 6. Segmentation of U2OS cells from the BBBC039 dataset [30]. The microbeSEG segmentation model (default settings) has been trained on HeLa cells from the Cell Tracking Challenge [31, 32].



(a) Image-segmentation overlay (frame 46) (b) Analysis results opened in Fiji

Fig 7. Exemplary microbeSEG segmentation (a) and analysis results (b) for a growing *C. glutamicum* colony. The results can easily be viewed in Fiji.

Tracking Challenge [31, 32]. Even without annotated U2OS cells, a good segmentation quality is achieved.

Data analysis

For time-lapse experiments, basic analysis results, i.e., cell counts, mean cell area, mean cell minor/major axis length and total area can be exported after segmentation. Possibly needed manual corrections to reach a virtually error-free segmentation can be applied using ObiWan-Microbi. The results can easily be plotted using Fiji [33] as Fig 7 shows.

Feature comparison

Table 2 provides an overview of crucial features that we believe a cell segmentation tool should have for ease of use, and also shows which software meets these features. Some tools support only a limited number of data formats, which results in additional conversion steps before the software can be used. In addition, most tools use no data management system that can store, visualize, and share data, metadata, and results. However, the support of such a data management system and its corresponding file format standards facilitates the use of segmentation tools. So far, most deep

Table 2. Cell segmentation software key feature comparison. Considered are only tools with graphical user interface since an end user should not need programming expertise. Non deep learning segmentation methods may require expert knowledge for parametrization and are not state-of-the-art anymore. Data format support does not necessarily mean that each image can be processed if no data management system (DMS) with metadata support is used, e.g., the channel dimension can be the first or the last dimension for .tif files and the method may have requirements on the channel dimension position. ▬: feature not fulfilled/supported, ⊘: feature only fulfilled/supported with restrictions, ✔: feature fulfilled/supported.

Software	DMS	Data formats	Deep learning	Cropping	Annotation	Pre-labeling	Training	Result corr.
AutoCellSeg [34]	▬	.jpg, .png, .tif, .bmp	▬	▬	▬	▬	▬	✔
BacStalk [35]	▬	.jpg, .tif	▬	▬	▬	▬	▬	▬[a]
Cellpose/Omnipose [12, 13, 36]	▬	.gif, .jpg, .png, .tif	✔	▬	✔	✔	✔	✔
ChipSeg [10]	▬	.tif	▬	▬	▬	▬	▬	▬
DeepImageJ [37] (Fiji plugin [33])	▬	>150[b]	✔	▬	▬	▬	▬	▬
Misic [15] (napari plugin [38])	▬	>150[b]	✔	▬	⊘[c]	▬	▬	▬
Orbit [39]	✔[d]	>150[b]	⊘[e]	▬	✔	▬	⊘[f]	▬
StarDist [40] (napari plugin [38])	▬	>150[b]	✔	▬	⊘[c]	▬	⊘[g]	▬
YeaZ [41]	▬	25	✔	▬	▬	▬	▬	✔
YeastSpotter [42]	▬	N.A.	✔	▬	▬	▬	▬	▬
microbeSEG	✔[d]	>150[b]	✔	✔	✔[h]	✔	✔	✔[h]

[a] *Only deletion of objects, no shape refinement or correction of false negatives, merges and split objects.*
[b] *OME Bio-Formats support (napari needs the napari-aicsimageio plugin: https://www.napari-hub.org/plugins/napari-aicsimageio).*
[c] *In principle possible with napari (plugins) but no training functionality with the graphical user interface.*
[d] *OMERO.*
[e] *With copy and paste of a segmentation script into the script editor: https://www.orbit.bio/deep-learning-object-segmentation/*
[f] *Only with a Python script outside of Orbit.*
[g] *Not within the graphical user interface, but in principle within the napari IPython console.*
[h] *With the associated tool ObiWan-Microbi.*

learning software with graphical user interface focuses on the application of pre-trained models and model training is not directly possible and requires programming expertise. Furthermore, training data annotation can require the use of incompatible annotation tools leading to further conversion steps. microbeSEG is the only tool which covers all key features (together with the jointly developed ObiWan-Microbi).

Discussion

Utilizing ObiWan-Microbi for training data annotation, microbeSEG covers the whole pipeline from training data creation, training, evaluation and inference. Both tools are designed to work together seamlessly and use OMERO for the data management. The optimized workflow allows producing high-quality segmentation results in reasonable annotation time. Our results indicate that switching to pre-label corrections is a better strategy than annotating manually when suitably accurate models are trained, since more cells can be annotated per time. In our experiments we observed, that it is a good strategy to correct mainly object level errors, i.e., merges, splits, and added or missing

cells. The AJI+ scores for *B. subtilis* are mainly limited by size differences and different handling of cell division events, as the qualitative segmentation results show. This is due to different decision boundaries of the microbeSEG user and the individual expert annotators in this experiment, especially for the cell division events of the challenging filamentous *B. subtilis* cells and for the *E. coli* size. We assume that when training and test data are annotated from the same person the AJI+ scores rise significantly.

The Omnipose segmentation results show that available software solutions may need retraining even when they are pre-trained on a large dataset, e.g., to avoid the segmentation of background structures on a microfluidic device. However, such solutions can be utilized to further reduce the manual annotation time. For instance, ObiWan-Microbi includes Omnipose pre-labeling. Prerequisite is that Omnipose works for the present data. A drawback is, however, that object shapes may not be matched as good as with manual annotation or the microbeSEG pre-labeling which enables to learn the object size decision boundary of the annotator.

Once a large and diverse annotated dataset is established, further annotation and model training is required only for significant changes in the experimental setup or for specific cases where the segmentation fails. Thereby, the microbeSEG crop selection with pre-labeling allows adding exactly those new training data where segmentation errors occur. A drawback of the training data crop creation is that for large image sizes, a small selected crop size and low object density, all proposed crops of a frame could show no cells, e.g., in a $2048\,\text{px} \times 2048\,\text{px}$ image with the smallest selectable crop size of $128\,\text{px} \times 128\,\text{px}$ and less than 10 objects with an object diameter of 10 px. In such a case, a larger crop size (crop sizes up to $1024\,\text{px} \times 1024\,\text{px}$ are available) needs to be selected. Another limitation of microbeSEG is that the segmentation method does not, like many others too, support the segmentation of overlapping objects.

In summary, we believe that the major concern about deep learning for cell segmentation, that extensive and time-consuming annotation of training data is required, has been overcome with our tool microbeSEG which provides an efficient workflow and an accurate segmentation method. Our segmentation software feature comparison has shown that no other segmentation tool provides all functionalities needed for the efficient segmentation of microbes. Certainly, microbeSEG can also be applied for the segmentation of cell nuclei or other objects. To further facilitate the data annotation for microbeSEG users, label inspection strategies [43] and training data simulators are of particular interest. A long-term goal is to leverage deep learning for 3D applications, e.g., to study plant-microbe interactions [44]. The integration of other state-of-the-art segmentation methods like Omnipose or StarDist into our tool and complete workflow are further possible future directions.

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