

DETECTION OF MICROORGANISMS IN COMPLEX MICROSCOPY IMAGES¹

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In this paper, we tackle the problem of finding microorganisms in bright field microscopy images, which is an important step in various tasks. Apart from bacteria or fungi, these images can contain impurities such as sand particles, which increases the difficulty of microbe detection. Following a semantic segmentation approach, where a label is inferred for each pixel, we achieve encouraging classification results on a database containing five different types of microbes.

Introduction

In many applications and research studies, analyzing microorganisms in images from bright field microscopes offers important insights. To derive quantitative results, biological researchers often put a lot of effort in labeling and counting bacteria in those images, which can be an exhausting task. This leads to only a small number of images used for evaluation and therefore to numbers which are doubtful from a statistical point of view.

Previous work on bacteria detection and cell segmentation has been done by Wu and Shah [1], who utilize a pixel-wise Conditional Random Field for binary cell segmentation making use of colored fluorescence images and an additional multi-spectral data source. In contrast, Gelas et al. [2] employ multi-phase level set segmentation for 3D cell segmentation using a Gaussian shape prior. Their main assumption is an already existing, very accurate initial segmentation provided by an expert. Seeded watershed transform is used by [3] for segmenting and tracking cells. All of these previous works assume that input images do not include impurities, such as sand particles, which is often not the case for real-world images (Fig. 1).

This work focuses on evaluating different state-of-the-art *semantic segmentation techniques* for the task of automatically detecting

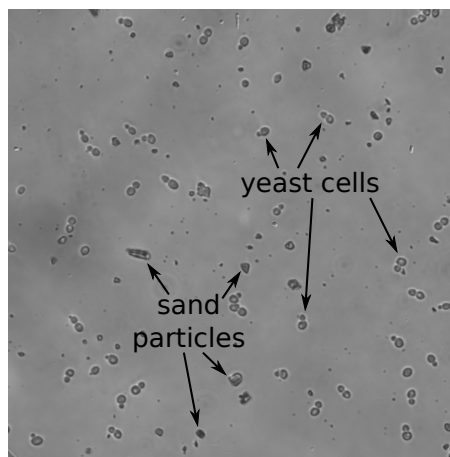


Fig. 1. Bright field microscopy image of yeast cells including sand particles and suffering from non-uniform illumination.

bacteria and other microbes. The benefit of semantic segmentation approaches is the availability of a label for each pixel, instead of a coarse labeling with a bounding box. Therefore, we analyze pixel-based level set segmentation and approaches based on segment classification and present how to incorporate application-specific prior knowledge such as shape information to distinguish cells from sand particles.

Our experiments show that incorporating shape and appearance analysis, as well as modeling label dependencies between segments using Conditional Random Fields, substantially reduces the false positive rate of our detection scheme and gives more useful results compared to a level set segmentation approach. This is es-

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pecially useful for input images which include a large number of outlier objects.

Segment Classification

The task of inferring a label for each pixel in an image has been considered important in a lot of different tasks, such as scene understanding [4] and facade recognition [5]. In the following, we shortly outline a simplified variant of [5], which is subsequently used for microbe detection.

The first step is to obtain a segmentation of the image. This can be done fully unsupervised by employing some clustering algorithm such as mean shift [6]. This is followed by a pixelwise classification on a predefined $M \times M$ -grid using a classifier previously learned on a training set. For each segment, a label can then be inferred by averaging the classification results for all grid points falling inside of a segment. Since shape and appearance of microbes play a crucial role in distinguishing them from background, we include a post-processing step which takes these properties into account. First of all, segments are labeled as background if they are too small (< 5 pixels) or too big (> 2000 pixels) in size. Secondly, shape and appearance features \mathbf{x}_i are extracted from the remaining segments and used for a rating in a second classification stage. In our experiments, a probabilistic Parzen classifier was used since it allows for a fast leave-one-out estimation. To obtain a hard decision, a segment i is classified as microbe if the probability $p(y_i = 1 | \mathbf{x}_i)$ of being microbe exceeds an automatically learned threshold. We used the minimal leave-one-out probability of all segments in the training image for this purpose. To take into account a possible over-segmentation of the image, neighboring segments are greedily clustered as to increase the segment likelihood of being microbe based on shape and appearance features.

Incorporating Structure with Conditional Random Fields

In order to globally take into account interdependencies, the heuristic and greedy merging strategy from the last section is often insufficient. However, it is often sensible to deviate from the common independence assumption $p(\mathbf{y} | \mathbf{X}) = \prod_{i=1}^n p(y_i | \mathbf{x}_i)$ and to include dependencies between segments. This

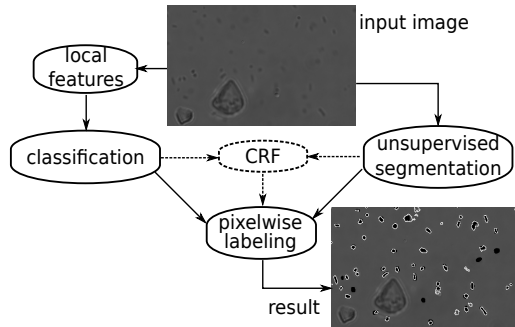


Fig. 2. Outline of our algorithm using segment classification and optional CRF optimization.

can be done using graphical models where a graph $G = (V, E)$ is designed to capture certain dependencies between random variables. While the nodes $\nu \in V$ represent these random variables (segment labels), direct dependencies between two variables are described by edges $e \in E$. Conditional Random Fields (CRFs) are a famous subclass of graphical models which allow us to formulate dependencies between labels $\mathbf{y} = (y_1, \dots, y_n)^T$ given some image features $\mathbf{X} = (\mathbf{x}_1, \dots, \mathbf{x}_n)^T$. It is known that for such an undirected dependency graph G , the joint probability of \mathbf{y} factorizes w.r.t. maximal cliques c in G , i.e. $p(\mathbf{y} | \mathbf{X}) = Z^{-1} \prod_c \Psi_c(\mathbf{y}_c | \mathbf{X}) = \exp(\sum_c \log \Psi_c(\mathbf{y}_c | \mathbf{X}) - \log Z)$. One way to do inference with respect to labels \mathbf{y} given observations \mathbf{X} , is to perform maximum a posteriori estimation. For sub-modular energies $\log p(\mathbf{y} | \mathbf{X})$, this can be efficiently done using Graph-Cut [7]. A standard way is to specify the log-potential functions $\log \Psi_c(\mathbf{y}_c | \mathbf{X})$ manually and we refer to this method as *Manual CRF* in our experiments.

Parameter Estimation for Log-linear CRFs

Manual specification of a CRF is a tricky task and can lead to models that do not generalize well on unseen images. An alternative is to linearly decompose the log-potential functions in a parametric label term $\theta(\mathbf{y}_c)$ and a feature term $f_c(\mathbf{X})$, i.e. $\log \Psi_c(\mathbf{y}_c | \mathbf{X}) = \theta(\mathbf{y}_c)^T f_c(\mathbf{X})$. By doing this, parameters $\theta(\mathbf{y}_c)$ can be learned in these log-linear CRFs by maximum likelihood estimation. Since the log-partition function $\log Z$ depends on those newly introduced parameters, the exact procedure is intractable and one has to resort to approximate infer-

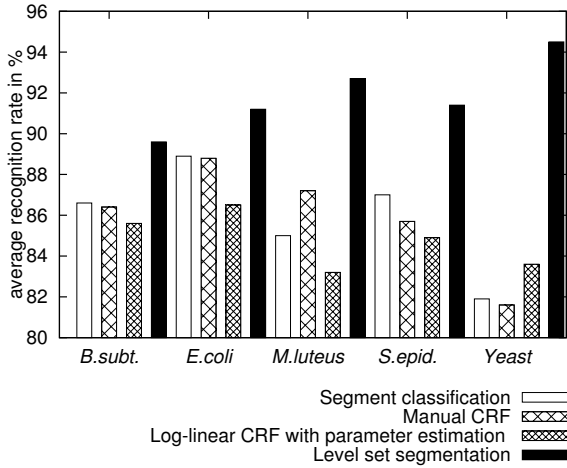


Fig. 3. Average recognition rate achieved by all methods for each of the bacteria types.

ence techniques such as loopy belief propagation (LBP). Learned parameters can then be used for inference, where again Graph-Cut can be employed. However, instead of using MAP estimation given an image \mathbf{X}_* , we infer the marginal probabilities $p(y_i^* = 1 | \mathbf{X}_*)$ of region i belonging to a microbe, using LBP. A hard decision can then be derived by thresholding those marginals. In this work, the threshold is empirically set to the 10th percentile of all marginal probabilities inferred for the training segments which corresponds to an assumed outlier ratio of 10%.

Level Set Segmentation

Active contours are curves that minimize a predefined energy functional which often consists of a data term preferring strong image edges and a regularization term favoring smooth contours. Level sets as first proposed by Osher and Sethian [8] can be understood as an extension to active contours, which can directly handle complex morphology changes such as splitting and merging of object boundaries. We used a binary Mumford-Shah model with Gaussian intensity distribution and geodesic active contour penalty. More details will not be documented in this abstract for reasons of space. For further information, we refer to [9]. As this method is pixel-based, no pre-processing as in previous sections can be performed.

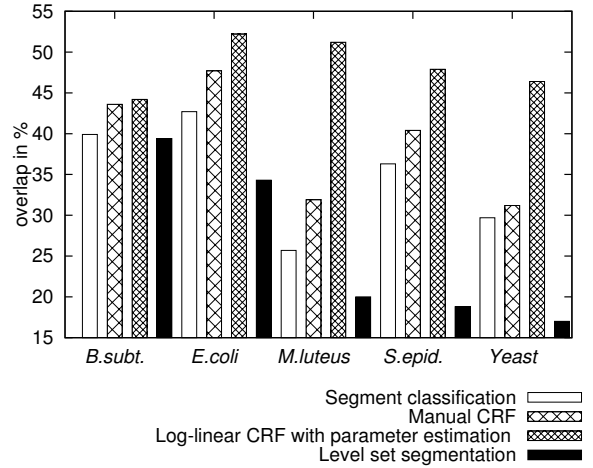


Fig. 4. Overlap ratio achieved by all methods for each of the bacteria types.

Experiments

In the following, methods presented in previous sections are utilized for pixel-wise microbe detection and evaluated on real-world images.

Performance Assessment We use a database including 5 different microbe species with 40 up to 470 microbes per class for training and testing.

While the same parameters are used for all microbe categories, recognition accuracy is measured within those categories. As performance measures, average recognition rate as well as the overlap between inferred microbe labeling and ground-truth microbe labeling are used. In our setting, a single image is used for training and another one for testing. To arrive at the final results, all measures are averaged over all possible combinations of training-testing pairs within each category.

Implementation Details For semantic segmentation as presented in the second section, pixel-wise classification is done using a decision tree based Gaussian process classifier [10]. The resulting pseudo-likelihood on the segment level is also used as unary potential of the manual CRF, where pairwise potentials are proportional to the average edge strength between two neighboring segments. For the log-linear CRF, gray values, thresholded intensity values, and an integrity measure are used as input features \mathbf{X} . Inference was done with the UGM package of Mark Schmidt [11].

Discussion of Results Final results are displayed in Fig. 3 and Fig. 4. It becomes apparent

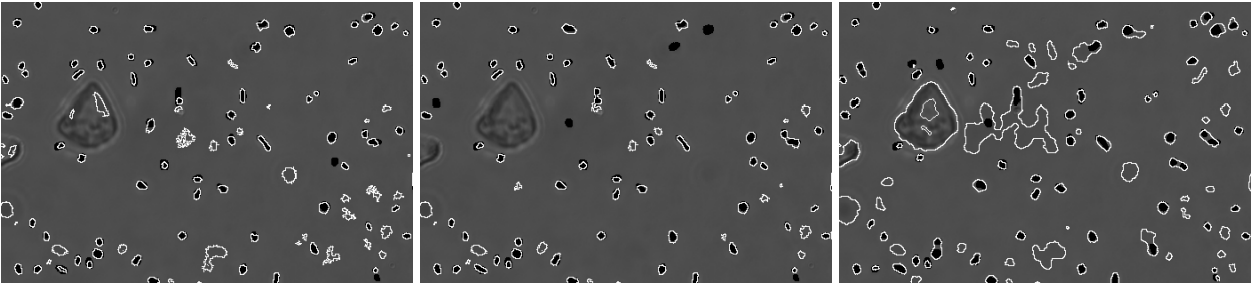


Fig. 5. Segmentation result using (left) intensity-based segment classification [4] with post-processing; (center) employing Graph-Cut based dependencies between segments; (right) and using a binary Mumford-Shah level set segmentation with Gaussian intensity distribution and geodesic active contour penalty. Ground-truth regions are filled with black color and the automatic results are shown by white contours.

that there is no method outperforming all other ones for all measures and categories. However, the fraction of false positives can be clearly reduced when dependencies between segments are taken into account. This can be also seen in Fig. 5, where example results are visualized for the microbe class *E.coli*. While the CRF-based approach labels less segments wrongly as bacteria, the level set approach leads to many false positives. Since for the latter, predicted microbe patches often include both classes, a post-processing step is often counterproductive. Thus, for applications that require a low number of false positives, CRFs should be preferred.

Conclusions and Further Work

This paper focused on an in-depth analysis and evaluation of several computer vision and machine learning techniques applied to the task of pixel-wise microbe detection. We compared several semantic segmentation techniques such as segment-based classification using Conditional Random Fields and region-based level set segmentation. In our opinion, the results of this paper can serve as a good guideline to select suitable algorithms for microbe segmentation which allow for learning from specific datasets.

Future research will focus on studying active learning methods to reduce the manual effort needed to label training images. Another interesting topic would be a comparison of different approximation methods for parameter estimation in Conditional Random Fields.

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