YeastFormer: An End-to-End Instance Segmentation Approach for Yeast Cells in Microstructure Environment

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- Abstract: Cell segmentation is a crucial task, especially in microstructured environments commonly used in synthetic biology. Segmenting cells in these environments becomes particularly challenging when the cells and the surrounding traps share similar characteristics. While deep learning-based methods have shown success in cell segmentation, limited progress has been made in segmenting yeast cells within such complex environments. Most current approaches rely on traditional machine learning techniques. To address this challenge, the study proposed a transfer-based instance segmentation approach to tackle both cell and trap segmentation in microstructured environments. The attention-based mechanism in the model's backbone enables a more precise focus on key features, leading to improved segmentation accuracy. The proposed approach outperforms existing state-of-the-art methods, achieving a 5% improvement in terms of Intersection over Union (IoU) for the segmentation of both cells and traps in microscopic images.

SCIENCE AND TECHNOLOGY PUBLIC ATIONS

1 INTRODUCTION

Yeast cells have been studied for decades in life sciences due to their well-characterized genome, membrane-bound organelles (like other eukaryotic cells), genetic traceability, ease of gene manipulation, availability, and overall simplicity of use(Lee, 2021). In 1957, the ultrastructure of yeast cells was first explored(Osumi, 2012), and since then advancements in imaging and molecular techniques have significantly improved our ability to study them. The classical baker's yeast, *Saccharomyces cerevisiae*, is one of the most widely used hosts for homologous and heterologous biopharmaceutical synthesis, protein production, and gene manipulation(Martínez et al., 2012). Beyond basic research, yeast cells are extensively

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used in industrial biotechnology, playing a crucial role in fermentation processes for brewing, baking, biofuel production, and winemaking(Onyema et al., 2023). In recent years, yeast cell research has gained important in various fields, including genetic engineering, pharmaceutical research, synthetic biology, and food science.

Data obtained from microscopes provide significant insights into various biological processes. Time-lapse fluorescence microscopy (TLFM) is an advanced tool that allows the investigation of dynamic cellular processes in living, intact cells(Nasser and Boudier, 2019). The extensive, standardized, and quantitative data generated by TLFM help advance our understanding of biomolecular functions and serve as a valuable resource for designing accurate and advanced biomolecular systems. A typical TLFM experiment using high-throughput microfluidics can generate thousands of specimen images, making manual annotation and segmentation a challenging task(Mahmoud, 2019). To address this, various automated segmentation techniques have been developed.

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Cell segmentation is a critical step in biomedical microscopy image analysis (Long, 2020). After accurate cell segmentation, several downstream tasks can be performed, such as cell tracking (Scherr et al., 2020), (Lugagne et al., 2020; Wen et al., 2021), cell counting (Loh et al., 2021; Ferreira and Silveira, 2024), cell type classification (Witmer and Bhanu, 2018), and cell phenotype analysis (Pratapa et al., 2021), among others. In modern microscopy studies, automated segmentation is essential for highthroughput analysis. Numerous approaches have been proposed to perform cell segmentation (Khalid et al., 2024; Durkee et al., 2021; Edlund et al., 2021; Khalid et al., 2023). However, it remains a challenging task due to factors such as varying cell shapes, overlapping cells, and inconsistent intensity levels in microscopic images. The difficulty is compounded in complex environments like microstructured environments, where precise control over mechanical properties, spatial arrangement, and chemical gradients is possible. Moreover, it is crucial to distinguish cells from other structures, such as debris or traps, particularly in the configurations discussed in this paper, where the appearance of the cells and traps is quite similar.

In this paper, a transformer-based network is proposed for cell-trap segmentation. The proposed pipeline, YeastFormer, utilizes ViTDet (Li et al., 2022) as the backbone and Cascade Mask R-CNN (Cai and Vasconcelos, 2019) for instance-based segmentation. ViTDet is responsible for feature extraction, while Cascade Mask R-CNN manages instancelevel segmentation tasks. A key strength of the model lies in its capability to effectively extract both finegrained local details and global contextual information, which are essential for accurate cell-trap segmentation. A detailed explanation of the proposed pipeline can be found in Section 3. Additionally, An Anchor-based ResNeSt (Edlund et al., 2021) model was fine-tuned and tested on the dataset. It was pretrained on the LiveCell dataset, one of the largest cell segmentation datasets, and the other on the COCO dataset. The proposed method outperformed it on cell segmentation, further demonstrating its robustness and effectiveness across various evaluations. The major contributions of this paper include:

- Proposing a robust transformer-based network that combines ViTDet (Li et al., 2022) for feature extraction and Cascade Mask R-CNN (Cai and Vasconcelos, 2019) for instance segmentation.
- Achieving a 5% improvement in IoU compared to state-of-the-art techniques.
- Demonstrating the efficacy of the model by comparing it with other state-of-the-art techniques in cell segmentation.

2 LITERATURE REVIEW

Many approaches have been proposed for cell detection and segmentation using both traditional computer vision methods (Al-Hafiz et al., 2018; Mohammed et al., 2013; Salem et al., 2016; He et al., 2022; Mandvartha et al., 2020) and deep learning-based techniques (Khalid et al., 2023; Wang et al., 2023; Khalid et al., 2022; Wang et al., 2022a). Traditional computer vision approaches typically employ methods such as intensity thresholding, region-based accumulation, and deformable model fitting. However, these approaches often rely on manual feature extraction tailored to specific tasks, which limits their generalizability. In contrast, deep learning (DL) approaches have significantly advanced cell segmentation by offering a data-driven methodology that requires less domain-specific expertise. A major breakthrough occurred with the introduction of U-Net (Ronneberger et al., 2015), which won the ISBI 2015 cell tracking and segmentation challenge. This innovation has greatly advanced biomedical research, leading to the development of tools such as DeepCell (Van Valen et al., 2016), CellPose (Stringer et al., 2021), Omnipose (Cutler et al., 2022), and Usiigaci (Tsai et al., 2019).

Several approaches have also been proposed for segmenting yeast cells in microscopic images (Salem et al., 2021; Kruitbosch et al., 2022; Kong et al., 2020; Lugagne et al., 2020; Haja and Schomaker, 2022). Studying yeast cells in microstructured environments allows for a tightly controlled setup, ensuring that the cells remain within the microscope's focal plane. Researchers have extensively investigated yeast cells in such environments (Liu et al., 2020; Gao et al., 2020; Wang et al., 2022b).

Deep learning techniques have also been applied to study cells in microstructured environments (Lugagne et al., 2020; Tognato et al., 2023). In (Prangemeier et al., 2020), the authors proposed a simple and faster attention-based cell detection transformer (CellDETR), which performs instance segmentation in microstructured environments. CellDETR achieves comparable results to Mask R-CNN, with a cell class Jaccard index of 0.84, but with fewer parameters and faster inference times. Synthetic data generation for yeast cells in microstructured environments is discussed in (Reich et al., 2021), where a MultiStyle-GAN is proposed for synthetic data generation based on prior knowledge. In (Prangemeier et al., 2022), U-Net was used for segmentation, while Mask R-CNN is employed for instance segmentation of cells in complex microstructured environments.

3 METHODOLOGY

Figure 1 provides a system overview of the proposed pipeline for yeast cell segmentation. The proposed network consists of the ViT Detector (ViTDet) backbone, Regional Proposal Network(RPN), and Cascade Mask RCNN as the prediction head, respectively.

3.1 Backbone Network

The backbone of the network is responsible for extracting features from the input data. It serves as the core feature extractor, capturing both high-level and low-level features from the input data. ViT Detector(ViTDet) has been used as the backbone of the proposed network. ViT Detector (ViTDet) is a specialized version of the Vision Transformer (ViT) (Alexey, 2020), designed specifically for object detection tasks. In ViTDet, the input image is divided into patches, which are embedded into vectors and passed through a stack of transformer blocks to capture both contextual and spatial relationships in a scalable manner. Using a global attention mechanism, the backbone enhances feature extraction, producing highly refined feature maps. The final feature map is then fed into a Simple Feature Pyramid (SPF), a streamlined version of traditional Feature Pyramids (Lin et al., 2017), commonly used in object detection tasks to handle objects of varying sizes. In the SPF, a series of convolutions or deconvolutions is applied in parallel, generating multi-scale feature maps at scales of 1/32, 1/16, 1/8, 1/4 from an initial feature map at a scale of 1/16.

3.2 Regional Proposal Network(RPN)

The features extracted from the backbone are passed to the Region Proposal Network (RPN) (Ren et al., 2016). The RPN is typically a fully convolutional network (FCN), and its purpose is to identify regions where objects may exist. This is achieved by drawing anchor boxes on the input image and comparing them to the ground truth using Intersection over Union (IoU). If the IoU exceeds the 0.7 threshold, the anchor box is assigned to the foreground and linked to one of the ground truth boxes. If the IoU is less than 0.3, the anchor is considered background, otherwise, it is ignored. After determining the anchor boxes, the distance between the anchor boxes and the ground truth is calculated. At this stage, Non-Maximum Suppression (NMS) (Cai and Vasconcelos, 2018) is applied to retain only the best regions by removing overlapping or redundant proposals.

3.3 Prediction Head

Cascade Mask R-CNN (Cai and Vasconcelos, 2019) was used as the prediction head in the proposed model. This architecture is an extension of Cascade R-CNN, with an additional mask branch to improve pixel-level predictions. As a multistage network, Cascade Mask R-CNN refines predictions by progressively increasing the IoU threshold at each stage. In the first stage, an IoU threshold of 0.5 is applied, and predictions that have over 50% overlap with the ground truth are passed to the next stage. In the second stage, the output from the first stage is treated as new region proposals, and an IoU threshold of 0.6 is used to refine the predictions further. In the final stage, an IoU of 0.7 is applied to enhance accu-



Figure 1: System overview of YeastFormer. The input image is passed to the network and an instance segmentation mask is produced as output.



Figure 2: Sample images from the dataset: The top row shows the original images, and the bottom row shows the overlay of the masks on the original images. Here, represents the cells and, represents the trap instances.

racy even more. In the proposed methodology, the segmentation branch is added at the last stage of the Cascade R-CNN. The box head classifies the object within the ROI and fine-tunes the shape and position of the box. A small fully convolution neural network is used as the mask head to produce the segmentation mask in pixel to pixel-to-pixel manner to attain the instance segmentation mask.

4 DATASET

The dataset used in this study is designed for segmenting yeast cells within a microstructured environment(Reich et al., 2023). It consists of 493 densely annotated brightfield microscopic images obtained from various TLFM experiments. The dataset includes images with the most common yeast and trap configurations, such as multiple cells, empty traps, and single cells (with daughter cells). Two different geometries of traps are included: L-shaped and oval-shaped. Out of the 493 images, 398 contain regularly shaped traps, while the remaining 95 contain L-shaped traps. The number of cells per image ranges from zero to six. Figure 2 shows sample images alongside their corresponding ground truth masks.

To enhance the versatility of the dataset, a diverse range of variations is included, such as trap type, focal shift, illumination levels, debris, and yeast morphology. Cells and traps appear similar in the images because both have roughly circular shapes and characteristic lengths. The dataset also captures challenging edge cases, such as broken traps, which add complexity to the segmentation task. Different instances of cells and traps are marked with distinct colors, while the background covering areas without cells or traps, as well as debris and incomplete cells near the edges is represented in gray. Table 1 provides an overview of the number of images, as well as the instances of cells and traps, across the training, validation, and test sets. The dataset is publicly available¹.

Table 1: Distribution of images, cells, and traps across training, validation, and test sets in the yeast cell dataset.

Split	Images	Cells	Traps		
Train	296	536	528		
Validation	49	108	98		
Test	148	270	291		

5 EVALUATION METRICS

To evaluate the performance of the proposed network, we employed the Jaccard index, the Panoptic Qual-

¹Dataset link:

https://github.com/ChristophReich1996/ Yeast-in-Microstructures-Dataset ity (PQ) metric, and mean Average Precision (mAP) as evaluation metrics. These metrics were chosen because they comprehensively evaluate different aspects of segmentation quality, such as overlap accuracy, instance-wise segmentation, and class-specific precision.

The Jaccard index (Hancock, 2004), also known as Intersection over Union (IoU), was utilized to compare the model with state-of-the-art techniques. It measures the ratio of the intersection of pixels between the ground truth and the model output to their union. This metric provides a clear understanding of how well the predicted segmentation matches the actual segmentation. IoU is calculated using the formula shown in Equation 1, where X represents the ground truth and \hat{X} denotes the prediction. Additionally, IoU for specific classes is reported to facilitate direct comparisons with results from earlier approaches, further validating the model's performance.

$$IoU = \frac{X \cap \widehat{X}}{X \cup \widehat{X}} \tag{1}$$

We also employed the standard COCO evaluation protocol (Lin et al., 2014) for mean average precision (mAP). This metric evaluates model performance at multiple IoU thresholds, specifically mAP50 and mAP75, representing IoU thresholds of 50% and 75%, respectively. Additionally, results are reported for different object size ranges, namely mAPs (small objects) and mAPm (medium objects). This detailed evaluation ensures a nuanced understanding of the model's performance across varying object scales. The Panoptic Quality (PQ) metric (Kirillov et al., 2019) was used to evaluate instance segmentation. This metric is particularly well-suited for datasets that can be treated as a specific case of panoptic segmentation. In this context, the trap and cell are categorized as thing classes, while the background is considered the sole stuff class. Consequently, the instance segmentation predictions on this dataset can be effectively assessed using PQ.

$$PQ = \frac{\sum_{(p,g)\in TP} IoU(p,g)}{|TP|} \frac{|TP|}{|TP| + \frac{1}{2}|FP| + \frac{1}{2}|FN|}$$
(2)

In PQ metric $\frac{1}{|TP|}\sum_{(p,g)\in TP} IoU(p,g)$ is used to compute the mean Intersection over Union (IoU) for all matched predicted segments p and their corresponding ground truth segments g. The PQ measures both the instance-wise segmentation quality (SQ) and the recognition quality (RQ)in a panoptic segmentation context. The mean average precision (mAP) is calculated using the formula shown in Equation 3, where n is the number of classes and (AP_i) is the average

precision for the n classes.

$$mAP = \frac{1}{n} \sum_{i=1}^{i=n} (AP_i)$$
(3)

6 EXPERIMENATAL SETUP

We conducted our evaluation using a range of distinct data settings to comprehensively assess the performance, robustness, and limitations of segmenting cells in a microstructured environment. The images feature different types of traps, including L-shaped and regular oval-shaped traps, with the number of cells ranging from zero to six per image. Additionally, we trained several networks on the dataset and compared their performance to our network. We trained and tested the Mask R-CNN model, following the experimental setup from (Prangemeier et al., 2022). Mask R-CNN enhances Faster R-CNN (Ren et al., 2016) by integrating object detection with instance segmentation. ROIAlign improves segmentation accuracy by preserving spatial details, while FPN constructs a multi-scale feature pyramid to effectively handle objects of different sizes.

Furthermore, we trained and tested the pre-trained Anchor-based ResNeSt network. The Anchor-based ResNeSt network was pre-trained on the LiveCell dataset and utilized the aspect ratios of the COCO(Lin et al., 2014) dataset (0.5, 1.0, and 2.0), with two additional aspect ratios of 3.0 and 4.0. The pixel means and standard deviations were adjusted according to the specifications of the LiveCell dataset. To handle small objects effectively, anchor sizes of 8, 16, 32, 64, and 128, were implemented.

The Segment Anything Model (SAM) (Kirillov et al., 2023) was also fine-tuned and tested to perform automated segmentation without requiring prompts. The SAM model is a foundational model in computer vision, leveraging a Masked Autoencoder with a Vision Transformer for scalability. It features a Prompt Encoder that generates prompt embeddings and a Mask Decoder that maps image and prompt embeddings to the final segmentation mask. Drawing inspiration from Transformer segmentation models, SAM incorporates a learned output token embedding into the prompt embedding. This output token plays a key role in guiding the decoder by encapsulating critical information required for precise image segmentation. We inputted the default embeddings from the prompt encoder into SAM's mask decoder. Empirical results confirm the effectiveness of this straightforward approach (Zhang and Liu, 2023; Gu et al., 2024).

	Method	ΔΡ	A P50	AP75	APs	APm	Cell IoI	Tran IoU	PO	
to evaluate instance segmentation. The methods marked with an asterisk (*) utilized different data splits.										
con	paring IoU metrics for the cell and trap classes acro	oss mul	tiple seg	mentati	on met	hods. P	anoptic Qu	ality (PQ)	is inclu	ded
Tab	le 2: Segmentation Performance - Average Precis	ion (A	P) is rep	ported a	cross v	arious 1	IoU thresh	olds and a	ea rang	ges,

Method	AP	AP50	AP75	APs	APm	Cell IoU	Trap IoU	PQ
C-DETR A* (Prangemeier et al., 2020)	-	-	-	-	-	83.0	85.0	-
C-DETR B* (Prangemeier et al., 2020)	-	-	-	-	-	84.0	86.0	-
DISCO* (Prangemeier et al., 2020)	-	-	-	-	-	70.0	-	-
Mask R-CNN (SOTA) (Prangemeier et al., 2022)	65.4	98.4	85.4	56.5	71.1	84.0	89.0	-
Segment Anything (SAM)(Fine-tuned)(Kirillov et al., 2023)	44.8	94.0	83.8	47.8	50.0	63.1	89.9	88.1
Anchor-based ResNeSt(Edlund et al., 2021)	78.2	99.3	95.0	70.9	83.4	87.6	90.7	89.0
CellPose(Stringer et al., 2021)	44.4	94.5	82.2	-	-	-	-	-
YeastFormer (Proposed)	80.7	99.4	97.2	73.7	84.4	89.0	90.5	90.0

Note: CellPose does not provide classification capabilities to distinguish between different classes in a microstructure environment.

In our network, we employed aspect ratios of 0.5, 1.0, and 2.0, with the set of anchor sizes (32, 64, 128, 256, and 512), corresponding to these aspect ratios. We used the AdamW optimizer (Loshchilov, 2017) with an initial learning rate of 5×10^{-5} and a decay rate of 0.8. The model was trained for 6000 iterations using an NVIDIA GeForce RTX 4090 GPU. All networks were implemented using the PyTorch framework (Paszke et al., 2019). The best checkpoints are selected based on the validation loss.

7 RESULTS AND DISCUSSION

Table 2 presents the segmentation AP scores averaged across both classes. The proposed method outperformed both competing approaches, achieving an overall AP score of 80.7%. At IoU thresholds of 0.50 and 0.75, the model attained AP scores of 99.4% and 97.2%, respectively.

We provided a comparison with state-of-the-art methods i.e. Mask R-CNN(Prangemeier et al., 2022). We also compared our model's results with DISCO, Cell-DETR A, and Cell-DETR B (Prangemeier et al., 2020), as presented in Table 2. DISCO (Bakker et al., 2018) utilized traditional techniques, such as template matching, Support Vector Machines (SVM)(Suthaharan and Suthaharan, 2016), and active contours. In contrast, Cell-DETR A and Cell-DETR B employed an attention-based transformer for Cell-Trap instance segmentation. However, our approach's results are not directly comparable with these methods due to differences in the data splits used across the models. For the experiments, we also trained and tested the Anchor-based ResNeSt LIVE-Cell model (Edlund et al., 2021) and the Segment Anything Model (SAM).

The results presented in Table 2 show that our model not only outperformed the state-of-the-art Mask R-CNN model but also performed better in all experiments. Our model achieved the highest cell IoU of 89.0, outperforming Mask R-CNN by a margin of 5%. The automatic mode of the Segment Anything Model (SAM) was utilized without providing any prompts to perform segmentation. However, the results were suboptimal, indicating that SAM requires prompts for better performance. The use of prompts, however, requires the integration of expert knowledge. In contrast, our proposed method operates independently of prompts and consistently outperforms all compared models. Cellpose (Stringer et al., 2021) performs class-agnostic segmentation on the dataset used in this study, meaning it cannot distinguish between cell and trap instances. However, distinguishing between these two is a key objective of our study. The results of Cellpose are also reported in Table 2. The proposed method outperforms CellPose on the segmentation task by a wide margin of 36.3% in terms of AP. These results show the potential of our proposed approach for instance segmentation of cells and traps in microstructure environment. In the current setup, the Intersection over Union (IoU) for the cell holds greater significance than the IoU for the trap.

Sample segmentation results of the different methods are shown in Figure 3. The results demonstrate that our proposed model consistently outperforms other approaches in various challenging scenarios. The first column displays the results from the Mask R-CNN (SoTA) model, followed by the second column showing outputs from the Anchor-based ResNeSt model trained on the LiveCell dataset. The third column presents the results from the Segment Anything Model (SAM), while the fourth column features the predictions generated by our proposed method. Ground truth cell masks are depicted by solid blue lines, with the model's predictions shown as dotted orange lines. Similarly, solid green lines represent the trap ground truth, and dotted red lines correspond to the model's predictions. Our method achieves the best IoU for both the cell and trap classes across all images and performs well even near boundary areas. In row (a), it is evident that all methods



Figure 3: The inference results for several sample images in which our model performed sufficiently are presented. Ground truth cell masks are outlined in solid blue, while model predictions are marked by dotted orange lines. Solid green lines represent ground truth traps, and dotted red lines indicate the model's trap predictions.

struggle near the boundaries of cells and traps. Mask R-CNN fails to accurately detect both cells and traps, while the Anchor-based ResNeSt and SAM also face difficulties in boundary areas, leading to cell oversegmentation. In row (b), SAM incorrectly identifies an artifact as a cell, significantly lowering the IoU, and fails to accurately segment traps. In row (c), the other networks continue to struggle with boundary delineation, as SAM is still unable to draw precise cell boundaries and over-segments cells. Meanwhile, in row (d), the Anchor-based ResNeSt detects a cell in the trap area where no cell is present. These observations highlight the robustness and superior accuracy of our model in handling complex and diverse situations compared to other methods.

For analysis, we present the results of our model



Figure 4: The inference results for several sample images in which our model performed insufficiently are presented. Ground truth cell masks are outlined in solid blue, while model predictions are marked by dotted orange lines. Solid green lines represent ground truth traps, and dotted red lines indicate the model's trap predictions.

on samples where it did not perform well, as shown in Figure 4. The first column illustrates results from the state-of-the-art Mask R-CNN (SoTA) model, while the second column presents outputs from the Anchorbased ResNeSt model. The third column features predictions from the Segment Anything Model (SAM), and the fourth column showcases the outputs of our proposed approach. Ground truth cell masks are represented by solid blue lines, with model predictions depicted as dotted orange lines. Similarly, solid green lines denote trap ground truth, and dotted red lines represent trap predictions by the models. Both types of traps are included in the images, providing a comprehensive assessment of segmentation performance.

Our model demonstrates consistent difficulties with trap prediction, reflected in a low Intersection over Union (IoU) score for the trap class. In row (a), it is clear that our approach struggles to delineate the lower boundary of the cell at the image's edge, as well as the trap boundary. SAM also fails in this scenario, unable to detect the cell near the border of the image. In row (b), the issue persists, with our model struggling to accurately capture boundaries at the image edge. Row (c) depicts a scenario where no cell is present, leading to a cell IoU of 0 for all methods. Here, our model achieves the lowest trap IoU among the approaches. In row (d), the model oversegments the cell located between two traps and fails to detect the traps accurately, further highlighting its limitations in handling complex spatial arrangements.

Overall, our proposed approach faces challenges when cell boundaries are ambiguous or when edge cases occur near image borders. These limitations point to areas for potential improvement in segmentation accuracy and robustness. Linking the model's outputs to biologically meaningful metrics or insights, such as cell counts, size distributions, or spatial relationships, helps biologists directly interpret predictions in the context of their experimental hypotheses or workflows.

Vision Transformers are generally more computationally demanding than CNNs due to their selfattention mechanism, which calculates interactions between every pair of image patches (Maurício et al., 2023). This capability allows Vision Transformers to effectively capture global dependencies across the entire image. In contrast, CNNs utilize convolutional operations that scale more efficiently in terms of computational complexity, although they may struggle to capture global dependencies in larger images as effectively as Vision Transformers. This study demonstrated that fine-tuning Vision Transformers achieved better results compared to fine-tuning CNNs. However, this performance improvement came with a trade-off: an increase in inference time for Vision Transformers relative to CNNs.

8 CONCLUSIONS

In this research, we introduce a novel framework for instance, the segmentation of yeast cells within a microstructured environment using a transformer-based technique. Our proposed approach enables precise detection and segmentation of individual yeast cells and traps, even in situations where they share similar characteristics, allowing for improved analysis of cell morphology. Through extensive experimentation, we demonstrate that this framework successfully differentiates between instances of yeast cells and traps in microscopic images, achieving robust performance. To show the adaptability of the model to different in vivo and in vitro microstructured environments, we tested it on various trap types. The proposed technique holds significant potential to assist biologists in analyzing yeast cell behavior within controlled environments, providing valuable insights into cellular dynamics. Future work will aim to enhance model performance further, ensuring greater accuracy and reliability in diverse scenarios. Efforts will also focus on extending this method to more microstructured environmental settings, broadening its applicability to a wider range of experimental conditions. Additionally, reducing computational demands will be a key objective, making the approach more practical and accessible for real-world applications.

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REFERENCES

- Al-Hafiz, F., Al-Megren, S., and Kurdi, H. (2018). Red blood cell segmentation by thresholding and canny detector. *Procedia Computer Science*, 141:327–334.
- Alexey, D. (2020). An image is worth 16x16 words: Transformers for image recognition at scale. arXiv preprint arXiv: 2010.11929.
- Bakker, E., Swain, P. S., and Crane, M. M. (2018). Morphologically constrained and data informed cell segmentation of budding yeast. *Bioinformatics*, 34(1):88–96.
- Cai, Z. and Vasconcelos, N. (2018). Cascade r-cnn: Delving into high quality object detection. In *Proceedings of the IEEE conference on computer vision and pattern recognition*, pages 6154–6162.
- Cai, Z. and Vasconcelos, N. (2019). Cascade r-cnn: Highquality object detection and instance segmentation. *IEEE transactions on pattern analysis and machine intelligence*, 43(5):1483–1498.
- Cutler, K. J., Stringer, C., Lo, T. W., Rappez, L., Stroustrup, N., Brook Peterson, S., Wiggins, P. A., and Mougous, J. D. (2022). Omnipose: a high-precision morphology-independent solution for bacterial cell segmentation. *Nature methods*, 19(11):1438–1448.
- Durkee, M. S., Abraham, R., Clark, M. R., and Giger, M. L. (2021). Artificial intelligence and cellular segmentation in tissue microscopy images. *The American journal of pathology*, 191(10):1693–1701.
- Edlund, C., Jackson, T. R., Khalid, N., Bevan, N., Dale, T., Dengel, A., Ahmed, S., Trygg, J., and Sjögren, R. (2021). Livecell—a large-scale dataset for label-free live cell segmentation. *Nature methods*, 18(9):1038– 1045.

- Ferreira, E. and Silveira, G. (2024). Classification and counting of cells in brightfield microscopy images: an application of convolutional neural networks. *Scientific Reports*, 14(1):9031.
- Gao, Z., Xu, J., Chen, K., Wang, S., Ouyang, Q., and Luo, C. (2020). Comparative analysis of yeast replicative lifespan in different trapping structures using an integrated microfluidic system. *Advanced Materials Technologies*, 5(12):2000655.
- Gu, H., Dong, H., Yang, J., and Mazurowski, M. A. (2024). How to build the best medical image segmentation algorithm using foundation models: a comprehensive empirical study with segment anything model. arXiv preprint arXiv:2404.09957.
- Haja, A. and Schomaker, L. R. (2022). A fully automated end-to-end process for fluorescence microscopy images of yeast cells: From segmentation to detection and classification. In *Proceedings of 2021 International Conference on Medical Imaging and Computer-Aided Diagnosis (MICAD 2021) Medical Imaging and Computer-Aided Diagnosis*, pages 37–46. Springer.
- Hancock, J. (2004). Jaccard Distance (Jaccard Index, Jaccard Similarity Coefficient).
- He, F., Mahmud, M. P., Kouzani, A. Z., Anwar, A., Jiang, F., and Ling, S. H. (2022). An improved slic algorithm for segmentation of microscopic cell images. *Biomedical Signal Processing and Control*, 73:103464.
- Khalid, N., Froes, T. C., Caroprese, M., Lovell, G., Trygg, J., Dengel, A., and Ahmed, S. (2023). Pace: Point annotation-based cell segmentation for efficient microscopic image analysis. In *International Conference on Artificial Neural Networks*, pages 545–557. Springer.
- Khalid, N., Koochali, M., Leon, D. N. L., Caroprese, M., Lovell, G., Porto, D. A., Trygg, J., Dengel, A., and Ahmed, S. (2024). Cellgenie: An end-to-end pipeline for synthetic cellular data generation and segmentation: A use case for cell segmentation in microscopic images. In Annual Conference on Medical Image Understanding and Analysis, pages 387–401. Springer.
- Khalid, N., Koochali, M., Rajashekar, V., Munir, M., Edlund, C., Jackson, T. R., Trygg, J., Sjögren, R., Dengel, A., and Ahmed, S. (2022). Deepmucs: a framework for co-culture microscopic image analysis: from generation to segmentation. In 2022 IEEE-EMBS International Conference on Biomedical and Health Informatics (BHI), pages 01–04. IEEE.
- Kirillov, A., He, K., Girshick, R., Rother, C., and Dollár, P. (2019). Panoptic segmentation. In Proceedings of the IEEE/CVF conference on computer vision and pattern recognition, pages 9404–9413.
- Kirillov, A., Mintun, E., Ravi, N., Mao, H., Rolland, C., Gustafson, L., Xiao, T., Whitehead, S., Berg, A. C., Lo, W.-Y., et al. (2023). Segment anything. In *Proceedings of the IEEE/CVF International Conference on Computer Vision*, pages 4015–4026.
- Kong, Y., Li, H., Ren, Y., Genchev, G. Z., Wang, X., Zhao, H., Xie, Z., and Lu, H. (2020). Automated yeast cells segmentation and counting using a parallel u-net based two-stage framework. OSA Continuum, 3(4):982–992.

- Kruitbosch, H. T., Mzayek, Y., Omlor, S., Guerra, P., and Milias-Argeitis, A. (2022). A convolutional neural network for segmentation of yeast cells without manual training annotations. *Bioinformatics*, 38(5):1427– 1433.
- Lee, B. H. (2021). Advanced Fermentation and Cell Technology, 2 Volume Set. John Wiley & Sons.
- Li, Y., Mao, H., Girshick, R., and He, K. (2022). Exploring plain vision transformer backbones for object detection. In *European conference on computer vision*, pages 280–296. Springer.
- Lin, T.-Y., Dollár, P., Girshick, R., He, K., Hariharan, B., and Belongie, S. (2017). Feature pyramid networks for object detection. In *Proceedings of the IEEE conference on computer vision and pattern recognition*, pages 2117–2125.
- Lin, T.-Y., Maire, M., Belongie, S., Hays, J., Perona, P., Ramanan, D., Dollár, P., and Zitnick, C. L. (2014). 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.
- Liu, P., Liu, H., Yuan, D., Jang, D., Yan, S., and Li, M. (2020). Separation and enrichment of yeast saccharomyces cerevisiae by shape using viscoelastic microfluidics. *Analytical Chemistry*, 93(3):1586–1595.
- Loh, D. R., Yong, W. X., Yapeter, J., Subburaj, K., and Chandramohanadas, R. (2021). A deep learning approach to the screening of malaria infection: Automated and rapid cell counting, object detection and instance segmentation using mask r-cnn. *Computerized Medical Imaging and Graphics*, 88:101845.
- Long, F. (2020). Microscopy cell nuclei segmentation with enhanced u-net. *BMC bioinformatics*, 21(1):8.
- Loshchilov, I. (2017). Decoupled weight decay regularization. arXiv preprint arXiv:1711.05101.
- Lugagne, J.-B., Lin, H., and Dunlop, M. J. (2020). Delta: Automated cell segmentation, tracking, and lineage reconstruction using deep learning. *PLoS computational biology*, 16(4):e1007673.
- Mahmoud, L. N. K. (2019). A dictionary-based denoising method toward a robust segmentation of noisy and densely packed nuclei in 3D biological microscopy images. PhD thesis, Sorbonne Université.
- Mandyartha, E. P., Anggraeny, F. T., Muttaqin, F., and Akbar, F. A. (2020). Global and adaptive thresholding technique for white blood cell image segmentation. In *Journal of Physics: Conference Series*, volume 1569, page 022054. IOP Publishing.
- Martínez, J. L., Liu, L., Petranovic, D., and Nielsen, J. (2012). Pharmaceutical protein production by yeast: towards production of human blood proteins by microbial fermentation. *Current opinion in biotechnol*ogy, 23(6):965–971.
- Maurício, J., Domingues, I., and Bernardino, J. (2023). Comparing vision transformers and convolutional neural networks for image classification: A literature review. *Applied Sciences*, 13(9):5521.
- Mohammed, E. A., Mohamed, M. M., Naugler, C., and Far, B. H. (2013). Chronic lymphocytic leukemia cell segmentation from microscopic blood images using wa-

tershed algorithm and optimal thresholding. In 2013 26th IEEE Canadian Conference on Electrical and Computer Engineering (CCECE), pages 1–5. IEEE.

- Nasser, L. and Boudier, T. (2019). A novel generic dictionary-based denoising method for improving noisy and densely packed nuclei segmentation in 3d time-lapse fluorescence microscopy images. *Scientific reports*, 9(1):5654.
- Onyema, V., Amadi, O., Moneke, A., and Agu, R. (2023). A brief review: Saccharomyces cerevisiae biodiversity potential and promising cell factories for exploitation in biotechnology and industry processes-west african natural yeasts contribution. *Food Chemistry Advances*, 2:100162.
- Osumi, M. (2012). Visualization of yeast cells by electron microscopy. *Journal of electron microscopy*, 61(6):343–365.
- Paszke, A., Gross, S., Massa, F., Lerer, A., Bradbury, J., Chanan, G., Killeen, T., Lin, Z., Gimelshein, N., Antiga, L., et al. (2019). Pytorch: An imperative style, high-performance deep learning library. Advances in neural information processing systems, 32.
- Prangemeier, T., Reich, C., and Koeppl, H. (2020). Attention-based transformers for instance segmentation of cells in microstructures. In 2020 IEEE International Conference on Bioinformatics and Biomedicine (BIBM), pages 700–707. IEEE.
- Prangemeier, T., Wildner, C., Françani, A. O., Reich, C., and Koeppl, H. (2022). Yeast cell segmentation in microstructured environments with deep learning. *Biosystems*, 211:104557.
- Pratapa, A., Doron, M., and Caicedo, J. C. (2021). Imagebased cell phenotyping with deep learning. *Current* opinion in chemical biology, 65:9–17.
- Reich, C., Prangemeier, T., Françani, A. O., and Koeppl, H. (2023). An instance segmentation dataset of yeast cells in microstructures. In 2023 45th Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC), pages 1–4. IEEE.
- Reich, C., Prangemeier, T., Wildner, C., and Koeppl, H. (2021). Multi-stylegan: Towards image-based simulation of time-lapse live-cell microscopy. In Medical Image Computing and Computer Assisted Intervention–MICCAI 2021: 24th International Conference, Strasbourg, France, September 27–October 1, 2021, Proceedings, Part VIII 24, pages 476–486. Springer.
- Ren, S., He, K., Girshick, R., and Sun, J. (2016). Faster r-cnn: Towards real-time object detection with region proposal networks. *IEEE transactions on pattern* analysis and machine intelligence, 39(6):1137–1149.
- Ronneberger, O., Fischer, P., and Brox, T. (2015). Unet: Convolutional networks for biomedical image segmentation. In Medical image computing and computer-assisted intervention–MICCAI 2015: 18th international conference, Munich, Germany, October 5-9, 2015, proceedings, part III 18, pages 234–241. Springer.
- Salem, D., Li, Y., Xi, P., Phenix, H., Cuperlovic-Culf, M., and Kaern, M. (2021). Yeastnet: Deeplearning-enabled accurate segmentation of budding

yeast cells in bright-field microscopy. *Applied Sciences*, 11(6):2692.

- Salem, N., Sobhy, N. M., and El Dosoky, M. (2016). A comparative study of white blood cells segmentation using otsu threshold and watershed transformation. *Journal of Biomedical Engineering and Medical Imaging*, 3(3):15.
- Scherr, T., Löffler, K., Böhland, M., and Mikut, R. (2020). Cell segmentation and tracking using cnn-based distance predictions and a graph-based matching strategy. *PLoS One*, 15(12):e0243219.
- Stringer, C., Wang, T., Michaelos, M., and Pachitariu, M. (2021). Cellpose: a generalist algorithm for cellular segmentation. *Nature methods*, 18(1):100–106.
- Suthaharan, S. and Suthaharan, S. (2016). Support vector machine. *Machine learning models and algorithms for big data classification: thinking with examples for effective learning*, pages 207–235.
- Tognato, R., Bronte Ciriza, D., Maragò, O., and Jones, P. (2023). Modelling red blood cell optical trapping by machine learning improved geometrical optics calculations. *Biomedical Optics Express*, 14(7):3748–3762.
- Tsai, H.-F., Gajda, J., Sloan, T. F., Rares, A., and Shen, A. Q. (2019). Usiigaci: Instance-aware cell tracking in stain-free phase contrast microscopy enabled by machine learning. *SoftwareX*, 9:230–237.
- Van Valen, D. A., Kudo, T., Lane, K. M., Macklin, D. N., Quach, N. T., DeFelice, M. M., Maayan, I., Tanouchi, Y., Ashley, E. A., and Covert, M. W. (2016). Deep learning automates the quantitative analysis of individual cells in live-cell imaging experiments. *PLoS computational biology*, 12(11):e1005177.
- Wang, C.-W., Huang, S.-C., Lee, Y.-C., Shen, Y.-J., Meng, S.-I., and Gaol, J. L. (2022a). Deep learning for bone marrow cell detection and classification on wholeslide images. *Medical Image Analysis*, 75:102270.
- Wang, Y., Wang, W., Liu, D., Hou, W., Zhou, T., and Ji, Z. (2023). Genesegnet: a deep learning framework for cell segmentation by integrating gene expression and imaging. *Genome Biology*, 24(1):235.
- Wang, Y., Zhu, Z., Liu, K., Xiao, Q., Geng, Y., Xu, F., Ouyang, S., Zheng, K., Fan, Y., Jin, N., et al. (2022b). A high-throughput microfluidic diploid yeast longterm culturing (dylc) chip capable of bud reorientation and concerted daughter dissection for replicative lifespan determination. *Journal of Nanobiotechnology*, 20(1):171.
- Wen, C., Miura, T., Voleti, V., Yamaguchi, K., Tsutsumi, M., Yamamoto, K., Otomo, K., Fujie, Y., Teramoto, T., Ishihara, T., et al. (2021). 3deecelltracker, a deep learning-based pipeline for segmenting and tracking cells in 3d time lapse images. *Elife*, 10:e59187.
- Witmer, A. and Bhanu, B. (2018). Multi-label classification of stem cell microscopy images using deep learning. In 2018 24th International Conference on Pattern Recognition (ICPR), pages 1408–1413. IEEE.
- Zhang, K. and Liu, D. (2023). Customized segment anything model for medical image segmentation. arXiv preprint arXiv:2304.13785.