CellBinDB: A Large-Scale Multimodal Annotated Dataset for Cell Segmentation with Benchmarking of Universal Models

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Abstract

In recent years, advances in cell segmentation techniques have played a critical role in the analysis of biological images, especially for quantitative studies. Deep learning models have shown remarkable performance in segmenting cell and nucleus boundaries, but are often designed for specific modalities or require human intervention to select hyper-parameters, and are limited in generalizing to out-of-sample data. Building universal cell segmentation models can address the above challenges, but requires a large amount of multimodal training data. Here, we present CellBinDB, a large-scale multimodal annotated dataset established for cell segmentation. CellBinDB contains more than 1,000 annotated images of DAPI, ssDNA, H&E, and mIF staining, covering more than 30 normal and diseased tissue types from human and mouse samples. Based on CellBinDB, we benchmarked six state-of-the-art cell segmentation models and a widely used software. Evaluations were performed on the entire dataset and on each staining type, with Cellpose performed outstandingly. In addition, we analyzed the effects of four cell morphology indicators and image gradient on the segmentation results.

Keywords: Cell Segmentation, Dataset, Universal Models, Benchmark

1 Introduction

Cell/nuclear staining technology enhances the visualization of cell or subcellular components in microscope images, thereby contributing to quantitative analysis in biomedical research[1]. As microscopy makes progress to study complex biological structures in great detail and produce abundant dense images[2], there is an urgent need for automated methods to extract cell information by accurate segmentation of cell and nuclei[1, 3]. Over the past decade, deep learning methods have been successfully demonstrated their outstanding of accurate nuclear or cell-boundary identification in limited latent features of some specific images[4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. However, selecting an appropriate strategy or pretraining models to segment cell/nuclear is not an easy task to non-expert users in regular biology labs. Recent efforts have focused on developing universally applicable image segmentation models for cell analysis[15, 16, 17]. Until now, universal cell segmentation still faces enormous challenges. The lack of diverse image datasets and high-quality annotations are the primary barriers hindering the development of general models[18, 19, 20, 21].

Previous efforts have released several public datasets for training deep learning models, although all important advances, they are limited in their ability to simultaneously meet the requirements for large-scale, multiple staining techniques, and diverse tissue types, thus unable to fulfill the development needs for universal models. Table 1 shows the annotated stained datasets that have been actively used by the research community in recent years. From the review in Table 1, it can be seen that some datasets are limited in scale or tissue type richness, such as MoNuSeg[18], IEEE_TMI_2019[22] and the fluorescence image dataset published by Kromp et al. in 2020[23], all have less than 100 images. A slightly

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larger datasets Lizard[24] only has 1 tissue type. It also can be seen from Table 1 that most of the mentioned datasets are based on H&E staining, since H&E staining is the most common type of staining in routine pathology[25]. Followed by immunofluorescence (IF) staining, which is more frequently employed in research settings. NuInsSeg[25] is limited to a single staining type of H&E, similarly, BBBC039[26] is limited to Hoechst, a membrane-permeable fluorescent dye. There are also three large-scale and more diverse datasets, the Data Science Bowl 2018 featured a dataset (BBBC038v1)[21] with 37,333 manually annotated cell nuclei, however, datasets commonly used to train and benchmark segmentation models in stained cell image are still much larger by comparison. Tissuenet[7] emphasizes immunostaining and lacks histological staining and nucleic acid staining. NeurIPS 2022 dataset[19] aims to achieve richness in image modalities, with constrained quantity in each image type, especially in the case of stained images.

Recently, many efforts have been reported that they are made toward universal methods and exhibit good robustness on unseen datasets[5, 6, 7, 9, 16, 17]. SAM[16] is a method based on the self-attention mechanism, claiming that it has capability on Zero-shot generalization of new image distributions and tasks. Cellpose1[5] is based on the U-Net architecture, which can precisely segment cells from a wide range of image types and does not require model retraining or parameter adjustments. Cellpose3[6] is an improved version, developed with a focus on out-of-box segmentation of noisy, blurred or under-sampled images. DeepCell[7] is a deep learning algorithm for accurate whole-cell segmentation that achieves human-level segmentation performance by combining a ResNet50 backbone network and a feature pyramid network. MEDIAR[17] stood out in the NeurIPS 2022 multimodal cell segmentation competition and achieved state of the art (SOTA). StarDist[9] uses star-convex polygons to represent cell shapes, allowing accurate cell localization even under challenging conditions, especially when dealing with overlapping cells. However, the diversity of currently available models and the inconsistency of segmentation quality metrics make it difficult to evaluate their relative performance based on literature descriptions[27]. Therefore, evaluating these universal algorithms on a new unseen annotated dataset is necessary.

In this study, we present a new large-scale, multimodal annotated dataset, CellBinDB, containing images from more than 30 human and mouse tissues with four staining types (DAPI, H&E, ssDNA, and mIF). Unlike previous efforts that may be limited in tissue type coverage[23, 24], CellBinDB includes a wide range of both normal and diseased tissues, making it one of the most comprehensive tissue-type datasets available. The dataset images come from the 10x Genomics platform and new data obtained from new experiments conducted by our research team. Manual annotation is a time-intensive task, which greatly limits the scale of datasets. Model annotation methods are affected by model style and cannot guarantee accuracy. To balance quality and efficiency in annotating this multimodal dataset, we used a combination of manual and semi-automatic annotation strategies. To eliminate potential biases in the reference model[28], a trained team of professional annotators manually revised the model segmentation results and then checked by experts, which means all annotations passed through two rounds of quality assurance. We make CellBinDB available to the research community and evaluate the performance of some general models on the dataset, providing recommendations for the selection of cell segmentation models in different scenarios.

Table 1: **Publicly available annotated segmentation datasets.**

Dataset	Staining type	Image tiles	Cell	organs	Tile size(pixels)
MoNuSeg[18]	H&E	44	28,846	9	1000 × 1000
IEEE_TMI_2019[22]	H&E	80	25,645	8	512 × 512 and 1000 × 1000
Kromp et al.[23]	immuno \DAPI	79	7,813	5	550 × 430 to 1360 × 1024
Lizard[24]	H&E	238	495,179	1	512 × 512
NuInsSeg[25]	H&E	665	30,698	31	512 × 512
BBBC038v1[21]	DAPI\Hoechst\H&E	841	37,333	unknown	256 × 256
BBBC039[26]	Hoechst	200	23,165	unknown	520 × 696
TissueNet[7]	immuno	3,200	1,300,000	9	512 × 512
NeurIPS 2022 cell segmentation competition dataset[1][15]	Jenner–Giemsa \immuno	713	134,710	unknown	512 × 512 to 4096 × 4096
CellBinDB	**DAPI \ssDNA \H&E \mIF**	**1044**	**109,083**	**35**	**256 × 256 or 512 × 512**

[1] NeurIPS 2022 cell segmentation competition dataset only counted stained images.

2 Results

2.1 Dataset

In this study, we propose CellBinDB, a dataset comprising 1,044 annotated microscopy images and 109,083 cell annotations across four staining types: DAPI, ssDNA, H&E, and mIF (Figure 1a). CellBinDB includes samples from two species, human and mouse, covering over 30 histologically diverse tissue types, including disease-relevant tissues (Figure 1b). The images in CellBinDB come from two sources: self-generated mouse images, and human images obtained from the open-access platform 10x Genomics. We annotate all images in CellBinDB and offer two types of image annotations: semantic and instance masks (Figure 1c). Annotation is a combination of manual and semi-automatic methods, with 60% manual annotation and the rest from semi-automatic annotation (Figure 1f). The annotation workflow is shown in Figure 1g, and all annotations double-checked by experts to ensure quality. Additional details are in the Methods section. CellBinDB has diverse features that make it suitable for training generalized segmentation models. To visualize the image features of CellBinDB, we applied t-distributed stochastic neighbor embedding (t-SNE)[29, 30] to neural network-learned image features, revealing clusters that generally align by staining type (Figure 1d). The ssDNA and DAPI staining types show feature similarities, while differences are evident between images of the same staining type from the two sources. Compared with previous datasets, which share overlapping staining types with CellBinDB, CellBinDB exhibits a wider feature distribution, encompassing features from most public datasets (Figure 1e). Supplementary Table 1 provides further details about CellBinDB.

2.2 Benchmark performance.

We evaluated several widely recognized segmentation models on CellBinDB, including specialized cell segmentation models: Cellpose1[5], Cellpose3[6], StarDist[9], DeepCell[7], MEDIAR[17, 15], SOTA model for general segmentation: SAM[16], and a software widely used in biomedicine and life sciences: CellProfiler[8]. Since different models were trained on different training sets, a fair test was conducted using the CellBinDB dataset, which none of the models had seen before. The evaluation on CellBinDB is divided into two steps: evaluation on the entire dataset and evaluation on each staining type.

2.2.1 Evaluation results on the entire dataset.

We first evaluated the segmentation capabilities of each model on multimodal cell images (Figure 2a). The experimental results show that except for CellProfiler and DeepCell, which performed poorly, the other models exhibited excellent performance. Among them, Cellpose3 performed the best and was the most recommended (Precision: 0.82, Recall: 0.61, F1 Score: 0.70, Dice: 0.72). In contrast, the performance of Cellpose1, which shares a similar architecture, was less outstanding, with a lower F1 average than Cellpose3. We believe this lower performance is due to the smaller and less diverse training dataset used for Cellpose1 compared to Cellpose3. Similarly, DeepCell exhibited poor overall performance because its training dataset lacks diversity and consists only of fluorescent staining images. Additionally, the machine learning-based CellProfiler performed the worst among the models evaluated.

2.2.2 Evaluation on four staining types respectively.

We next evaluate the segmentation capabilities of each model on four staining types respectively. Figure 1 illustrates the diverse characteristics of images in CellBinDB. The model evaluation on the four staining types highlights the varying levels of difficulty across the stains and each model's strengths. In detail, most models scored higher on DAPI (Figure 2b,f) and ssDNA (Figure 2c,g) stains. Notably, Cellpose1, DeepCell, and MEDIAR performed exceptionally well on these two stain types and are highly recommended. In contrast, significant variability was observed in model performance on H&E (Figure 2d,h) and mIF (Figure 2e,i) stains. On H&E-stained images, StarDist, SAM, and Cellpose3 performed well, whereas other models showed poor results or even failure. We attribute StarDist's superior performance to its specialized weights trained for H&E-stained images, while SAM and Cellpose3 benefited from their large training datasets, which allowed them to effectively segment H&E images. The poor performance of CellProfiler and DeepCell likely stems from their incorrect interpretation of the foreground and background (Figure 2h). mIF is a membrane stain that has the opposite color characteristics compared to DAPI and ssDNA, showing that the interior of the cell is black. On mIF-stained images, Cellpose1, Cellpose3, and MEDIAR are the most recommended. While SAM was able to handle mIF images, its performance was relatively poor. The remaining models failed to effectively perform cell segmentation on mIF images because they mistakenly segmented the bright cell membranes as cells. Overall, H&E-stained and mIF-stained images pose greater segmentation challenges compared to other stain types.

Considering the specificity of H&E-stained image, some models perform additional preprocessing of the image, such as converting RGB to grayscale, or color deconvolution[31], to make the image features more consistent with the

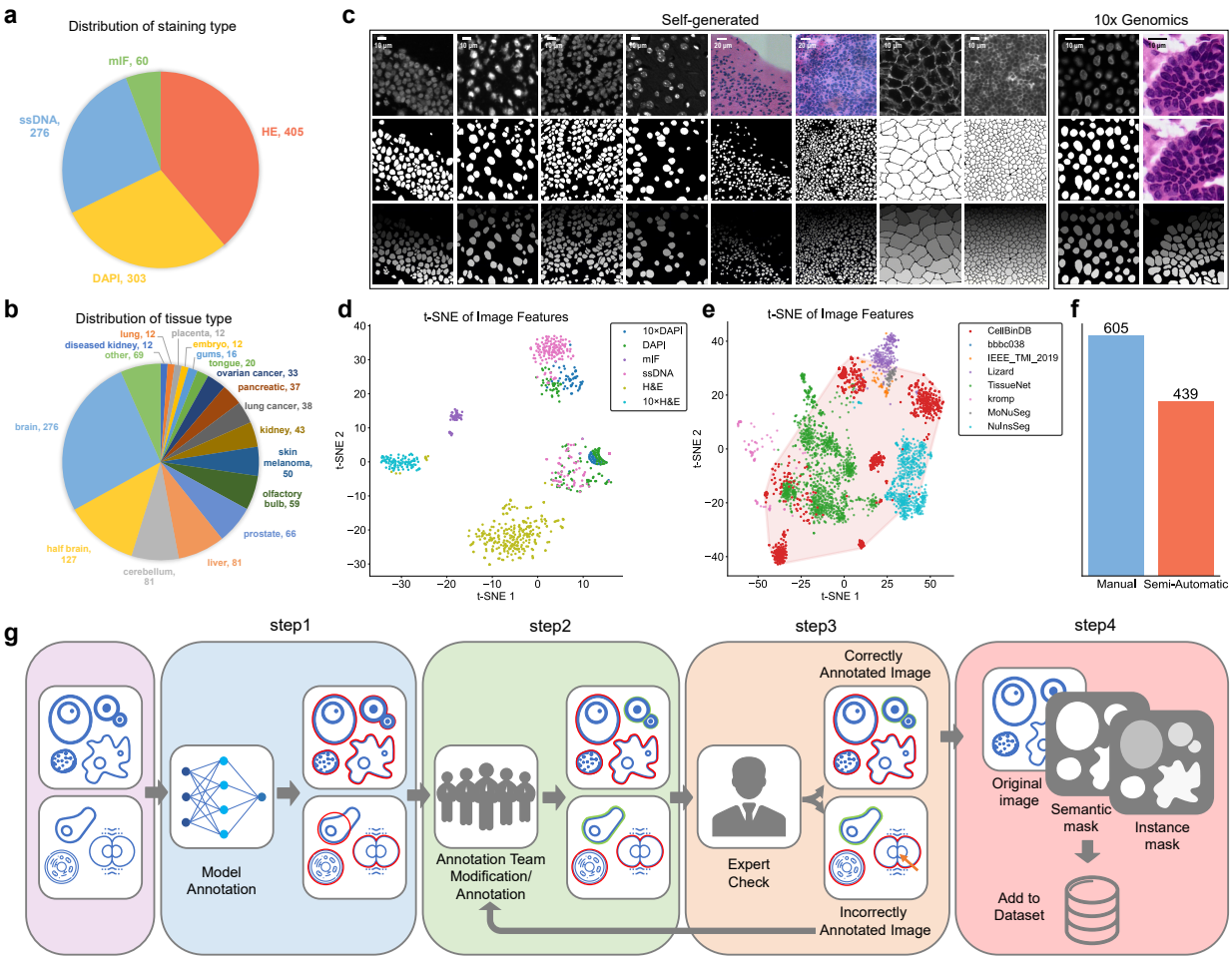

Figure 1: **Dataset overview.** a, Distribution of staining types in CellBinDB. b, Distribution of tissue types in CellBinDB, where tissue types with less than 10 samples are included in other, for details, see supplementary table 1. c, Examples of CellBinDB images and their two types of (semantic and instance) ground truth annotations, with 10 μm scale bar from left to right columns 1, 2: ssDNA, columns 3, 4: DAPI, columns 5, 6: H&E, columns 7, 8: mIF, column 9: 10x Genomics DAPI, column 10: 10x Genomics H&E. The first row is the original microscope image, followed by the semantic annotation mask and instance annotation mask. d, Scatter plot of t-SNE demonstrates the diverse spread of data by different staining types and sources. e, Scatter plot of t-SNE demonstrates the diversity of CellBinDB compared to previous datasets. f, The number of manual and semi-automatic annotations in CellBinDB. g, The dataset annotation process includes four steps: 1.model annotation, 2.annotation team modification/re-annotation(depends on the model annotation results), 3.expert review, go to the next step if the annotations are correct, otherwise return to the second step for modification, 4. add the original image and the two masks to the dataset.

a model capable of multimodal cell image segmentation is required without any presets or preprocessing, we recommend using SAM.

2.3 Factors affecting cell segmentation performance.

Next, we analyzed the factors that affect the model's ability to segment cells from both biological and non-biological perspectives.

2.3.1 Evaluation of impact of cell morphology.

We evaluated a series of cell morphology metrics, including the cell morphology evaluation metrics in BIDCell[32] and an original metric for evaluating cell density, cell average distance. We found that for CellBinDB, the four metrics

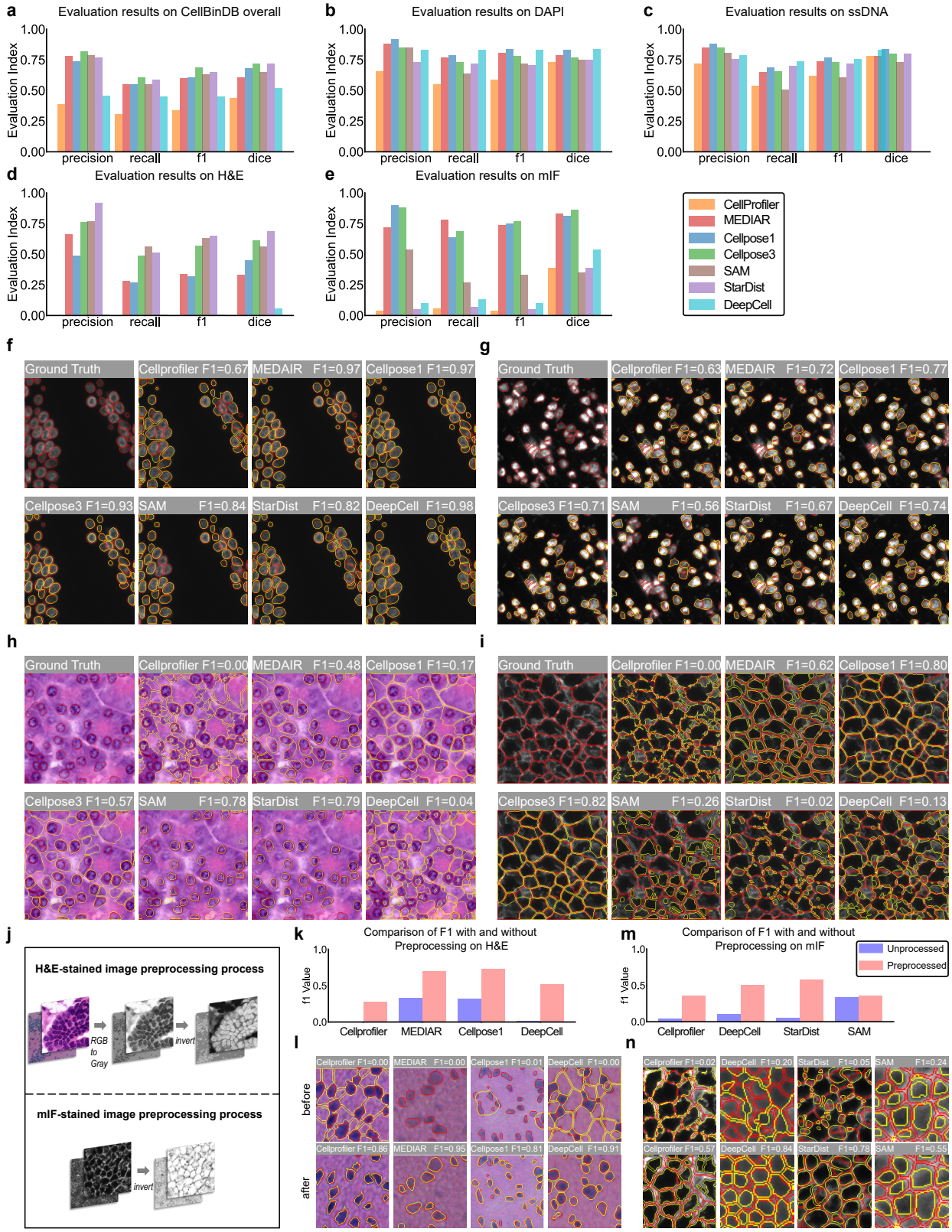

Figure 2: **Evaluation of model performance on the entire dataset and by staining type.**

a, Evaluate model performance on the entire dataset, with bar charts of precision, recall, F1 score, and dice. **b,** Model performance evaluation results on DAPI-stained images. **c,** Model performance evaluation results on ssDNA-stained images. **d,** Model performance evaluation results on H&E-stained images. **e,** Model performance evaluation results on mIF-stained images. **f-i,** Examples of segmentation results of seven cell segmentation models on four stainings (DAPI, ssDNA, H&E, mIF). **j,** H&E and mIF-stained image preprocessing process. H&E-stained images include two steps: grayscale conversion and color inversion, while mIF-stained only needs to perform color inversion. **k,** Comparison of F1 scores before and after adding the preprocessing process for the model with improved performance of H&E-stained images. **l,** The segmentation results of the four models with the worst segmentation results on the H&E-stained image before and after image preprocessing. **m,** Comparison of F1 scores before and after adding the preprocessing process for the model with improved performance of mIF-stained images. **n,** The segmentation results of the four models with the worst segmentation results on the mIF-stained image before and after image preprocessing.

most strongly correlated with the F1 score are cell area, average distance, cell circularity, and cell compactness. The experimental results indicate that larger cell area, greater distance between cells, and increased cell roundness and compactness are associated with better segmentation performance. Taking Cellpose1 as an example for fluorescence staining, which performed the best overall (Figure 3a,c), and StarDist as an example for H&E-stained (Figure 3b,d). The complete conclusions containing all the models are in the Supplementary Figure 1. The fluorescence staining images do not show a significant difference between low and medium on the indicator cellArea, which is possibly due to the characteristics of the data itself, with the majority of images having cell areas concentrated in the region with lower values.

Cell morphology is directly related to tissue type. We classify the images in the dataset according to tissue type, calculate the above four indicators, and then normalize them. These results are presented in Figure 3e, which provides an overview of the difficulty level of cell segmentation for different tissue types and indicates the potential challenges encountered in cell segmentation for each tissue type based on specific cellular features. Finally, we computed the mean value of the aforementioned four normalized cell morphology indicators. Based on these mean values, we stratified the image samples into low, medium, and high groups and further explored the correlation between the mean values of normalized cell morphology indicators and the F1 score. The results demonstrated that as the mean values increased, so did the F1 scores (Figure 3f). This finding indicates that the four selected indicators can, to a certain extent, reflect the difficulty of cell segmentation tasks.

2.3.2 Evaluation of Image Quality Factors on Model Segmentation Performance.

While morphological metrics are used to assess the impact of biological factors on samples, this study investigates the influence of image quality factors. Many deep learning models incorporate gradient information into their loss functions, such as the flow field in Cellpose1. This experiment aims to explore the effect of cell image gradients on model segmentation performance. The results indicate that, for CellBinDB, the gradient in cell images does indeed impact segmentation outcomes.

The relationship between the image gradient magnitude calculated by the sobel operator and the F1 score shows that most algorithms perform better on cell images of high-gradient magnitude, while their performance on low-gradient magnitude images deteriorates significantly (Figure 4a,b,c). This suggests that high-gradient magnitude images often provide more edge information, which helps cell segmentation models to perform better in accurately locating cell boundaries. At the same time, we also observed that the performance difference between high-gradient and medium-gradient images is relatively small. This may indicate that gradient variation is not the only factor determining segmentation performance; other factors such as noise, and the complexity of cell morphology may also play important roles. Thus, we conclude that higher gradients magnitude are beneficial to cell segmentation models as they assist in edge detection and feature extraction.

To validate the above conclusions, we conducted simulation experiments and applied varying degrees of Gaussian blur to ssDNA images to reduce the gradient magnitude (which are detailed in the Methods section).We generated cell images with different gradient characteristics using this method (Figure 4e), and grouped them into low, medium, and high gradient categories. Next, we applied all algorithms to segment the cells in these images and calculated the F1 scores for each algorithm across the different gradient groups. As shown in Figure 4d, the results indicated that for all algorithms, the low, medium, and high gradient groups corresponded to low, medium, and high F1 scores, respectively. This means that high-gradient cell images generally yielded better segmentation outcomes, while low-gradient images significantly impaired performance, leading to lower F1 scores.These experimental results further confirm our previous conclusions, namely that high-gradient cell images are easier to segment. By reducing the gradients of the images, we

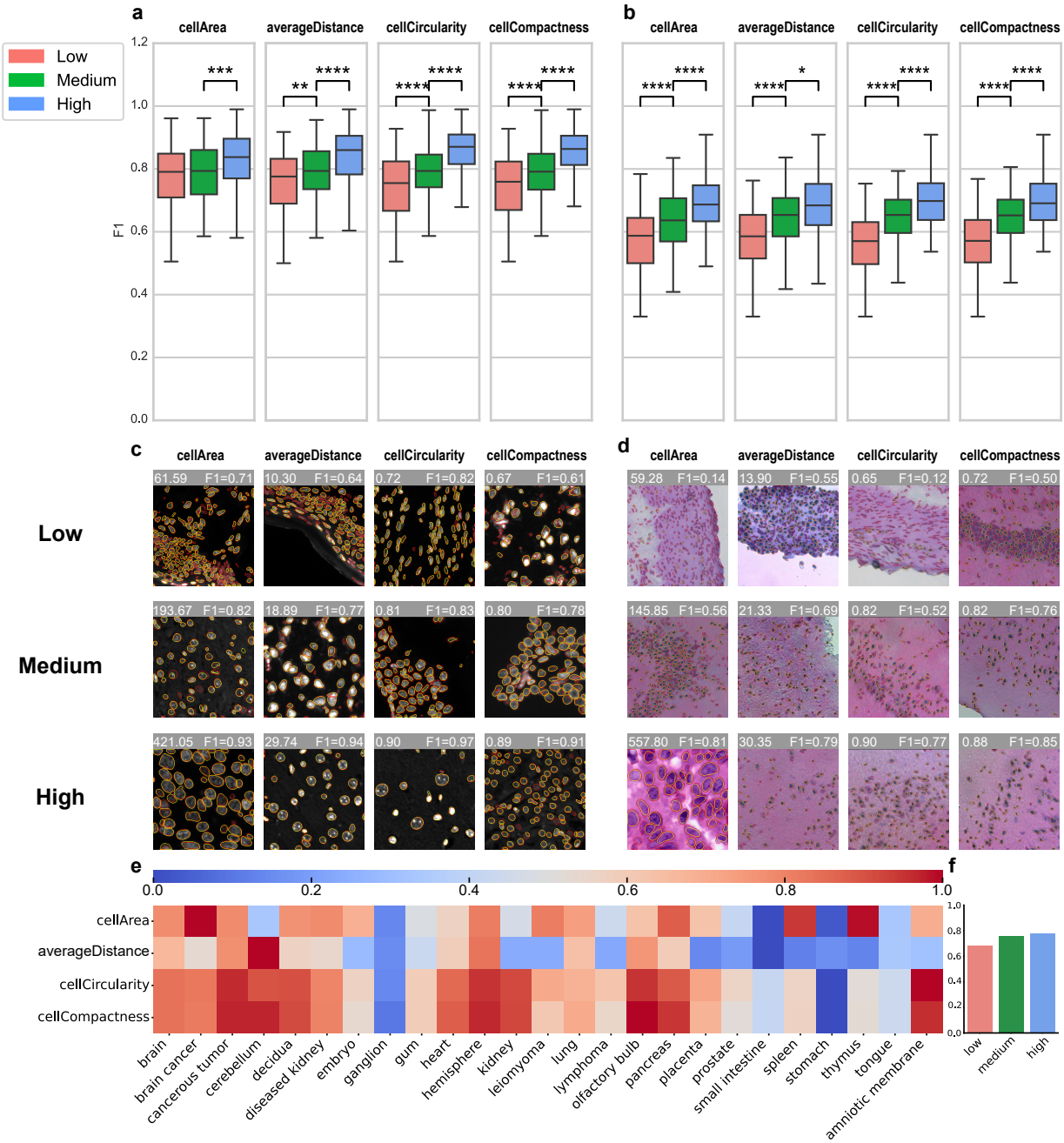

Figure 3: **Evaluation of the impact of cell morphology.** a, For fluorescent stained images (DAPI and ssDNA, Cellpose1 as an example), four indicators (cellArea, averageDistance, cellCircularity, cellCompactness) are used to evaluate the impact of cell morphology on segmentation performance. The vertical axis of the box plot is the F1 score, and the horizontal axis is the sample divided into three parts: low, medium and high according to the tertile of each indicator. And the significance mark line and p-value are added to the box plot. The number of "*" from 1 to 4 represents p-value less than 0.05, 0.01, 0.001 and 0.0001. b, Same as subfigure a, results on H&E-stained images. c, Fluorescent staining images display of low, medium and high instances under four indicators. d, H&E-stained images display of low, medium and high instances under four indicators. e, Display of normalized scores for the above four metrics by tissue type. f, According to the mean of the four cell morphology evaluation indicators of each image, it is divided into three groups: low, medium and high. The bar graph shows the relationship with the F1 score.

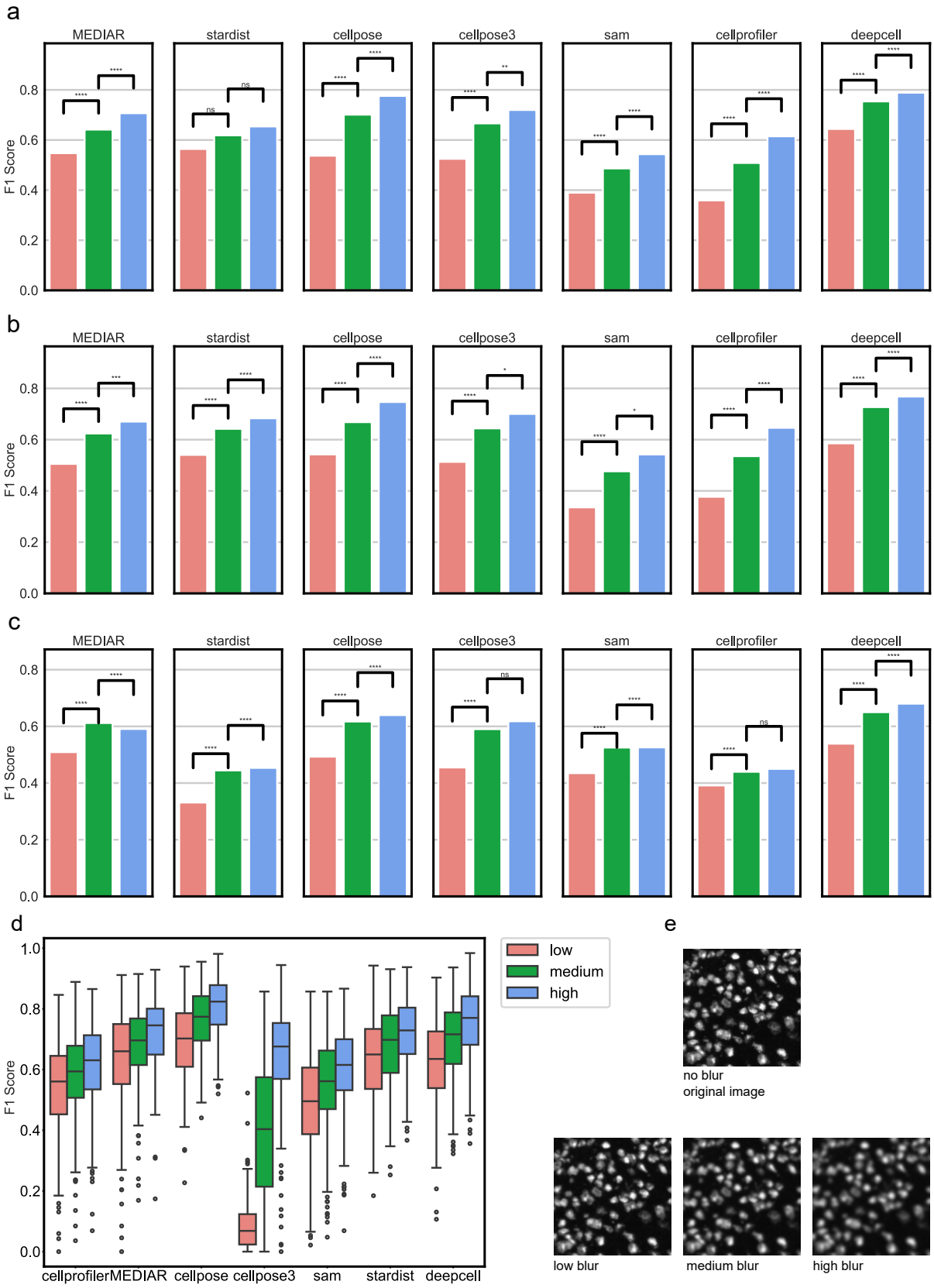

Figure 4: **F1 Score vs Cell Gradient Groups for Different Algorithms.**

a, DAPI-stained images, relationship between cell gradient and F1 score. The vertical axis of the box plot is the F1 score, and the horizontal axis is the sample divided into three parts: low, medium and high according to the tertile of cell gradient. And the significance mark line and p-value are added to the box plot. The number of "*" from 1 to 4 represents p-value less than 0.05, 0.01, 0.001 and 0.0001. **b,** Same as subfigure a, results on ssDNA-stained images. **c,** Same as subfigure a, results on H&E-stained images. **d,** Simulation Experiment Results. **e,** examples of low, medium and high Gaussian blurred images.

simulated varying degrees of edge information loss, and observed that low-gradient images resulted in a decrease in segmentation accuracy. This demonstrates the critical role of gradients in cell segmentation.

3 Discussion

In this paper, we introduced CellBinDB, a novel, comprehensive, and large-scale multimodal annotated dataset, aimed to enrich the existing cell segmentation data ecology and promote the development of universal cell segmentation models.

Based on CellBinDB, we evaluated the performance of cell segmentation models on a series of benchmark tasks. CellBinDB is completely new and unseen for all the models evaluated. We first evaluated the robustness and generalization of the cell segmentation models on the entire dataset. Our results show that without retraining and fine-tuning, the non-deep learning method CellProfiler has limited generalization ability and its performance significantly lags behind deep learning models. In stark contrast, deep learning models, especially Cellpose3, show strong segmentation and generalization performance, highlighting their superiority in this field. Considering that different staining types and the image features learned by different models in the training set vary greatly, we then evaluated the cell segmentation models on image subsets of four staining types separately. In this evaluation, we found the challenges of cell segmentation on H&E-stained images and mIF-stained images and proposed a solution. Based on the above cell segmentation tasks, we conducted a comprehensive evaluation of the model performance and provided model selection suggestions for different cell segmentation scenarios.

Subsequently, we explore the factors that affect cell segmentation results. First, from the biological factors of the samples, differences in cell morphology such as cell area, cell density, and roundness directly affect the difficulty of cell segmentation. We quantified this effect using four indicators that are positively correlated with the F1 score. Different tissue types are directly related to cell morphology. We provide the normalized scores of cell morphological indicators for each tissue in CellBinDB to quantify the difficulty of cell segmentation in different tissues. Through this evaluation, we can estimate the model performance by the tissue type of the image to be segmented. For example, we can expect that cell segmentation in tissues such as the small intestine, ganglion, and tongue will be challenging. Finally, the evaluation of the impact of image quality shows that high image gradient magnitude is beneficial for cell segmentation. Image noise, blur, etc. will affect image gradient, which may be due to the limitations of the optical system itself, the sample preparation process, and the uncertainty of imaging conditions. Therefore, laboratory personnel should strictly perform experimental operations to provide higher quality image data for downstream.

While this work presents contributions, it is important to acknowledge its limitations. The dataset utilized in this study, although diverse, is confined to two-dimensional (2D) images. In recent years, the prevalence of three-dimensional (3D) images has increased, posing new and unique challenges for cell segmentation tasks. To address these emerging challenges, researchers have already developed algorithms capable of performing 3D cell segmentation. Recognizing the importance of this advancement, we intend to delve into this direction and explore the potential of extending CellBinDB to incorporate 3D images in future research endeavors.

In conclusion, compared to any previous work, this work provides a contribution in dataset diversity and size, but this is only a small step in the direction of universal model development. While the comprehensive and extensive dataset presented in this work serves as a valuable resource for advancing cell segmentation models, it is crucial to recognize that further efforts are required to achieve true universality. The complexities and nuances within the field necessitate ongoing research and the continuous expansion of datasets to encompass various staining types, tissue types, and imaging modalities. By continuing to build upon this preliminary progress, we can collectively work towards the ultimate goal of developing models that can generalize and adapt to diverse datasets, staining techniques, and tissue types in the field of cell segmentation.

4 Methods

The code library of this study is implemented in Python3[33], using numpy[34], scipy[35], pandas[36], skimage[37], opencv[38] and sklearn[30], and using jupyter[39] to create a notebook version. The figures in this article were made using matplotlib[40] and seaborn[41], and formatted using Adobe Illustrator.

4.1 Data collection and annotation.

4.1.1 Image acquisition.

CellBinDB contains 30 mouse tissues and 5 human tissues. The 5 different human tissue samples are from the open platform 10x Genomics, with download links provided in the Data Availability section. For two IF-stained images (human skin melanoma and human prostate cancer), only the DAPI channel was selected. The mouse tissues in this study were collected from 6-week-old C57BL/6 both female and male laboratory mouses. All experimental protocols for generating dataset adhered to ethical regulations regarding animal research. Fresh frozen samples were prepared and sectioned as described in STOmics Stereo-seq Transcriptomics Set User Manual. Tissue slices of 10 μm were attached to the chip surface and stained after the tissue fixation. During the imaging stage, select the epi-bright field (color camera) mode for H&E-stained tissues and the epi-fluorescence mode for fluorescent-stained tissues. After completing the photography according to the manual requirements, the imageQC module of the StereoMap software was used for image quality control.

WSIs are generated by 1. a STOmics Microscope Go Optical equipped with Scanner Version 1.2.2, using 10×/0.75 NA and 20X/0.5 NA objective and Go Optical Scanner Ximea Mc124 for grayscale images and Go Optical Scanner Ximea Mc050 for RGB images. 2. a Motic PA53 FS6 Microscope equipped with PA53Scanner 1.0.0.14, using 10×/0.75 NA objective and PA53 FS6 SCAN S5LITE MONO for grayscale images and PA53 FS6 SCAN S5LITE for RGB images. We obtained whole slide images (WSIs) from the laboratory or open platforms and then cropped them to 512×512-pixel or 256×256-pixel sizes. A biologist selected the most representative fields of view (FOV) for each WSI, ensuring that the selected FOV images were clear and suitable for creating ground truth.

4.1.2 Image annotation.

We trained a model on CellBinDB and trained a team of professional annotators to semi-automatically annotate the dataset proposed in this study through three steps: model segmentation, manual modification or re-annotation, and expert verification. The model we used for annotation is from Li, M. et al.[28] The model architecture is psaUnet contained 5 encoder blocks and 5 decoders blocks, in the preprocessing stage, median filtering is used to smooth the noise that may exist in the input image, and in the post-processing stage, operations such as corrosion, dilation, and watershed are performed. The preliminary results of the model segmentation were saved in Tagged Image File Format (TIFF) and then converted to JavaScript Object Notation (json) format for import into Qupath 0.4.3 software[42]. In Qupath 0.4.3, the nuclei annotated by the model were visualized as polygons overlaid on the original nucleus images. If the model segmentation results were satisfactory, the annotation team obtained more accurate annotations by refining polygon outlines, deleting or adding polygons to modify the results of the model segmentation. If the model segmentation results were unsatisfactory, the annotation team re-annotated manually. The model we trained did not support cell membrane segmentation, mIF staining images could only be annotated manually, the model annotation step was skipped, and the annotation team directly used Qupath 0.4.3 software to outline the cells with the polygon tool. These annotations were then checked by experts. The incorrectly annotated images and corresponding modification suggestions were returned to the annotation team. Once the annotations were verified as correct, the two types of masks: instance mask and semantic mask, were exported and converted into TIFF. Finally, the microscope images were added to the dataset along with the two forms of masks.

The annotation of cell is challenging due to out-of-focus or nuclei presenting with modified morphology because of the slide preparation procedure. We defined the following criteria to annotate images:

- Naked eye is the highest standard, all cells that can be identified by naked eye are annotated, no false negatives (FN) or false positives (FP).
- The entire cell must be completely covered without any omissions or obvious gaps.
- The annotated cell boundaries should be smooth without excessive jagged edges.
- Overlapping cells should be annotated separately.
- The segmentation should not be too fragmented. For elongated cells, do not split them into multiple cells.

4.2 Model architecture and default parameters.

SAM[16] claims that it has capability on Zero-shot generalization of new image distributions and tasks. The model_type of SAM uses 'vit_b' and utilizes SamAutomaticMaskGenerator to automatically generate masks without the need for external prompts.

Cellpose1[5] can segment many types of cell without requiring parameter adjustments, new training data or further model retraining. Cellpose1 uses the 'cyto' model, with the channels for grayscale images set to [0,0] and for HE images set to [1,3]. The diameter is set to None.

Cellpose3[6] introduced a new method for biological image that achieves efficient segmentation without the requirement for clean images. Cellpose 3 uses the 'cyto3' model, with the channels for grayscale images set to [0,0] and for HE images set to [1,3]. The diameter is set to None, and the 'denoise_cyto3' model is used for noisy images.

DeepCell[7] achieves human-level accuracy across a variety of tissues and imaging modalities while requiring no manual parameter tuning from the end user. DeepCell uses the 'Mesmer' model, with image_map set to 0.5 and compartment set to 'nuclear'.

MEDIAR[17] stood out in the NeurIPS 2022 cell segmentation competition15 and achieved state of the art (SOTA). MEDIAR uses the provided from_phase2.pth model, with model_args configured as follows: 'classes' is set to 3, 'decoder_channels' is set to [1024, 512, 256, 128, 64], 'decoder_pab_channels' is set to 256, 'encoder_name' is set to 'mit_b5', and 'in_channels' is set to 3. The algo_params has 'use_tta' set to True.

StarDist[9] uses star-convex polygons to represent cell shapes, allowing accurate cell localization even under challenging conditions. StarDist uses the '2D_versatile_he' model for HE images and the '2D_demo' model for non-HE images.

Cellprofiler[8] is one of the earliest high-throughput cell image analysis platforms. Cellprofiler segmentation pipeline for non-H&E images: First, use IdentifyPrimaryObjects to identify cell objects, setting "Discard objects touching the border of the image" to No. Then, apply OverlayOutlines to outline the cells. Next, use ExpandOrShrinkObject to erode the cell boundaries, separating closely adjacent cells. Convert the image to a mask using MaskImage and finally, save the image with SaveImage. For H&E images, the segmentation pipeline is similar but starts with an additional step: convert the image to grayscale and then invert the colors before proceeding with the same steps as for non-H&E images.

4.3 Benchmark pipeline.

Different from some previous work[27, 43, 44, 45], we designed a series of benchmarks to test the performance of each model without retraining, maximizing the diversity and breadth of CellBinDB to evaluate the generalizability and robustness of each model. Unless otherwise specified, default parameters were used. Each step of the benchmark targets a different cell segmentation scenario:

(1) Model evaluation on the entire dataset. First, we test each model on the entire dataset including all staining types and tissue types and evaluate the overall performance results using the metrics precision, recall, F1 score, and Dice. The purpose of this step is to recommend the model with the best overall performance in cases where the user is segmenting a multimodal dataset or when the user lacks familiarity with their own data's attributes.

(2) Model evaluation on different staining types. In this step, the dataset is divided into four staining types and the performance of each model is tested individually on each single staining type. This step is intended to furnish users with model suggestions for cell segmentation reliant on specific stain types, as well as to facilitate a comparison of the relative challenges imposed by different staining types.

(3) Evaluating the impact of cell morphology on the performance of cell segmentation models. To measure cell morphology, we measured a series of metrics for each cell in the dataset, representing different cell characteristics. We then selected several metrics (cell area, average distance, cell circularity, and cell compactness) with the greatest impact on the F1 score to explore the relationship between cell morphology and model performance. Initially, the values for these four indicators were computed for each cell, and the mean value for each image was determined. Subsequently, the images were categorized into three groups—low, medium, and high—based on tertiles, to assess whether there are significant differences in the F1 scores across these groups. Given the similarity in features between DAPI and ssDNA-stained images, these two types were analyzed together. In contrast, H&E-stained images were analyzed separately, and mIF-stained images were excluded from this experiment due to their unique membrane staining characteristics.

(4) Exploring the effect of cellular image gradients on model segmentation performance. First, we matched individual cells, ground truth, and predicted results. Then, we calculated the gradient magnitude of each

cell using the Sobel operator in OpenCV, along with its corresponding F1 score. Subsequently, cells were categorized into three groups—low, medium, and high gradients—to assess whether there were significant differences in F1 scores across these groups.

4.4 Metrics.

4.4.1 Segmentation benchmark.

To evaluate models' performance on benchmark pipeline, an evaluation protocol was used which is calculated in several steps. First, the overlap between each prediction and its closest ground truth object is quantified using the IoU as follows:

$$\text{IoU} = \frac{P \cap G}{P \cup G} \tag{1}$$

where P is prediction, G is ground truth. If the IoU between the prediction and the closest ground truth object is greater than 0.5, the ground truth object is considered to be correctly segmented. For all ground truth objects, the segmentation performance is then quantified using the precision and recall metrics given below:

$$\text{precision} = \frac{TP}{TP + FP} \tag{2}$$

$$\text{recall} = \frac{TP}{TP + FN} \tag{3}$$

where TP is the number of true positives, FP is the number of false positives and FN is the number of false negatives. The F1 score is a metric used to assess the balance between precision and recall. It is the harmonic mean of precision and recall, providing a single score that takes both into account.

$$\text{F1 score} = \frac{2 \times (precision \times recall)}{precision + recall} \tag{4}$$

In addition, two pixel-level evaluation metrics are introduced:

$$\text{Dice} = \frac{2 \times |A \cap B|}{|A| + |B|} = \frac{2 \times TP}{2 \times TP + FP + FN} \tag{5}$$

4.4.2 Cytomorphological indicators.

To explore the impact of cell morphology on segmentation performance, we measured a series of morphological metrics for each cell in the dataset and selected the ones with the greatest impact, including cell area, average cell distance, cell circularity, and cell compactness.

The cell area is the number of pixels contained in each cell. The average cell distance is the average distance between each cell and the center point of the nearest cell. Cell circularity is used to calculate the circularity of the cell shape. The closer the value is to 1, the closer the cell shape is to a circle. We used the cv2.convexHull() function in OpenCV to get the convex hull of each cell, used the cv2.arclength function to get the convex hull area, and then calculated the circularity according to the following formula:

$$\text{Circularity} = \frac{4\pi \times \text{Area}}{\text{Convex.P}^2} \tag{6}$$

Compactness is the degree of compactness of the cell shape, calculated based on the cell area and cell perimeter. The closer the compactness is to 1, the more compact and circular the cell shape is; the closer the compactness is to 0, the more irregular and scattered the cell shape is.

$$\text{Compactness} = \frac{4\pi \times \text{Area}}{P^2} \tag{7}$$

The difference between the above two indicators is that cell roundness uses the perimeter of the cell convex hull, while compactness uses the cell perimeter. If there are many irregular protrusions or depressions on the edge of the cell, the value of compactness will obviously deviate from 1. Roundness pays more attention to the roundness of the overall shape and is less affected by slight irregularities on the edge.

4.4.3 Image gradient calculation.

We used the ground truth mask to identify the cell regions in the cell images, then applied the Sobel operator from OpenCV (cv2) to compute the x and y gradients of the cell images, followed by calculating the gradient magnitude and average.

The formula for the gradient in the x-direction (G_x):

$$G_x = I * S_x \tag{8}$$

where I is the input image, and S_x is the Sobel kernel matrix for the x-direction:

$$S_x = \begin{bmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{bmatrix} \tag{9}$$

The formula for the gradient in the y-direction (G_y):

$$G_y = I * S_y \tag{10}$$

where S_y is the Sobel kernel matrix for the y-direction:

$$S_y = \begin{bmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{bmatrix} \tag{11}$$

The gradient magnitude (G) is calculated as:

$$G = \sqrt{G_x^2 + G_y^2} \tag{12}$$

The average gradient magnitude is calculated as:

$$\text{Average Gradient Magnitude} = \frac{1}{N} \sum_{i=1}^{N} G_i \tag{13}$$

where N is the number of pixels in the image, and G_i is the gradient magnitude of each pixel.

4.5 Simulation experiments.

We used several different Gaussian blur parameters and adjusted the gradients of these blurred images to create three categories: low, medium, and high gradients. Each category of images had varying levels of blur, aimed at simulating images with different gradient magnitudes in order to assess the impact of gradient on cell segmentation performance. The blurring was implemented using OpenCV's GaussianBlur function. The parameters for low-intensity blurring were a (5,5) kernel size with sigmaX set to 1; for medium intensity, a (9,9) kernel size with sigmaX set to 2; and for high intensity, a (13,13) kernel size with sigmaX set to 3. After applying the blur, we calculated the average gradient magnitude of the images. The calculation formula for Gaussian blurring is as follows:

$$I_{\text{blur}}(x, y) = \sum_{i=-k}^{k} \sum_{j=-k}^{k} I(x+i, y+j) \cdot G(i, j) \tag{14}$$

Where I(x+i,y+j) is the original pixel intensity at position (x+i,y+j), G(i,j)is the Gaussian kernel weight for the position (i,j) relative to the center.

5 Data availability

The raw data of 5 human samples from the open platform 10×Genimics are available free of charge. The download links are as follows.

H&E human lung cancer: FFPE Human Lung Cancer with Xenium Multimodal Cell Segmentation.

H&E human pancreas: FFPE Human Pancreas with Xenium Multimodal Cell Segmentation.

H&E human ovarian cancer: FFPE Human Ovarian Cancer Data with Human Immuno-Oncology Profiling Panel and Custom Add-on.

DAPI human skin melanoma: Human Melanoma, IF Stained (FFPE).

DAPI human prostate Cancer: Human Prostate Cancer, Adjacent Normal Section with IF Staining (FFPE).

CellBinDB have been deposited into CNGB Sequence Archive (CNSA)[46] of China National GeneBank DataBase (CNGBdb)[47] with accession number CNP0006370.

6 Code availability

All the code used to produce the results reported in this article can be found at STOmics/cs-benchmark(github.com)

7 Authors' Contributions

Project administration and supervision: Mei Li, Ying Zhang

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8 Competing interests

The authors declare they have no competing interests.

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