

 Open access • Posted Content • DOI:10.1101/2021.06.05.447183

Dice-XMBD: Deep learning-based cell segmentation for imaging mass cytometry

— [Source link](#) 

[Xu Xiao](#), [Ying Qiao](#), [Yudi Jiao](#), [Na Fu](#) ...+4 more authors

Institutions: [Xiamen University](#)

Published on: 07 Jun 2021 - [bioRxiv](#) (Cold Spring Harbor Laboratory)

Topics: [Segmentation](#)

Related papers:

- [Dice-XMBD: Deep Learning-Based Cell Segmentation for Imaging Mass Cytometry.](#)
- [Robust microbial cell segmentation by optical-phase thresholding with minimal processing requirements.](#)
- [YeastNet: Deep-Learning-Enabled Accurate Segmentation of Budding Yeast Cells in Bright-Field Microscopy](#)
- [Automated cell boundary and 3D nuclear segmentation of cells in suspension](#)
- [Machine and deep learning single-cell segmentation and quantification of multi-dimensional tissue images](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/dice-xmbd-deep-learning-based-cell-segmentation-for-imaging-59ozh7jftq>

Dice-XMBD: Deep learning-based cell segmentation for imaging mass cytometry

Xu Xiao^{1,2,#}, Ying Qiao^{1,#}, Yudi Jiao¹, Na Fu¹, Wenxian Yang³,
Liansheng Wang^{1,*}, Rongshan Yu^{1,2,*} and Jiahuai Han^{2,4,*}

¹ Department of Computer Science, School of Informatics, Xiamen University, China

² National Institute for Data Science in Health and Medicine, Xiamen University, China

³ Aginome Scientific, Xiamen, China

⁴ School of Medicine, Xiamen University, China

These authors have contributed equally to this work.

* Corresponding authors {lswang, rsyu, jhan}@xmu.edu.cn

Abstract

Highly multiplexed imaging technology is a powerful tool to facilitate understanding cells composition and interaction in tumor microenvironment at subcellular resolution, which is crucial for both basic research and clinical applications. Imaging mass cytometry (IMC), a multiplex imaging method recently introduced, can measure up to 40 markers simultaneously in one tissue section by using a high-resolution laser with a mass cytometer. However, due to its high resolution and large number of channels, how to process and interpret the image data from IMC remains a key challenge for its further applications. Accurate and reliable single cell segmentation is the first and a critical step to process IMC image data. Unfortunately, existing segmentation pipelines either produce inaccurate cell segmentation results, or require manual annotation which is very time-consuming. Here, we developed Dice-XMBD, a Deep learnIng-based Cell sEGmentation algorithm for tissue multiplexed imaging data. In comparison with other state-of-the-art cell segmentation methods currently used in IMC, Dice-XMBD generates more accurate single cell masks efficiently on IMC images produced with different nuclear, membrane and cytoplasm markers. All codes and datasets are available at <https://github.com/xmuyulab/Dice-XMBD>.

Keywords: imaging mass cytometry, multiplexed imaging, single cell segmentation, U-Net, knowledge distillation, digital pathology

1 Introduction

Analysis of the heterogeneity of cells is critical to discover the complexity and factuality of life system. Recently, single-cell sequencing technologies have been increasingly used in the research of developmental physiology and disease [1, 2, 3, 4], but the spatial context of individual cells in the tissue is lost due to tissue dissociation in these technologies. On the other hand, traditional immunohistochemistry (IHC) and immunofluorescence (IF) preserve spatial context but the number of biomarkers is limited. The development of multiplex IHC/IF (mIHC/mIF) technologies has enabled the detection of multiple biomarkers simultaneously and preserve spatial information, such as cyclic IHC/IF and metal-based multiplex imaging technologies [5, 6, 7, 8]. Imaging mass cytometry (IMC) [6, 9], one of metal-based mIHC technologies, uses a high-resolution laser with a mass cytometer and makes the measurement of 100 markers possible.

39 IMC has been utilized in studies of cancer and autoimmune disorders [6, 10, 11, 12, 13]. Due
40 to its high resolution and large number of concurrent marker channels available, IMC has been
41 proven to be highly effective in identifying the complex cell phenotypes and interactions coupled
42 with spatial locations. Thus, it has become a powerful tool to study tumor microenvironment and
43 discover the underlying disease-relevant mechanisms [14, 15, 16, 17, 18, 19, 20, 21]. Apart from
44 using IMC techniques alone, several other technologies, such as RNA detection in situ and 3D
45 imaging, have been combined with IMC to expand its applicability and utility [22, 23, 24, 25].

46 The IMC data analysis pipeline typically starts with single-cell segmentation followed by
47 tissue/cell type identification [26, 27, 28]. As the first step of an IMC data processing pipeline,
48 the accuracy of single-cell segmentation plays a significant role in determining the quality and the
49 reliability of the biological results from an IMC study. Existing IMC cell segmentation methods
50 include both unsupervised and supervised algorithms. Unsupervised cell segmentation, such as
51 the watershed algorithm implemented in CellProfiler [26], does not require user inputs for model
52 training. However, the segmentation results are not precise when cells are packed closely or they
53 are in complicated shapes. To achieve better segmentation results, it is possible to use supervised
54 methods with a set of annotated images with pixel-level cell masks to train a segmentation classifier.
55 However, the manual annotation task is very time-consuming and expensive as well since it is
56 normally done by pathologists or experienced staff with necessary knowledge in cell annotation.
57 Particularly, for multiplexing cellular imaging methods such as IMC, their channel configurations
58 including the total number of markers and their selections are typically study-dependent. Therefore,
59 manual annotation may need to be performed repeatedly for each study to adapt the segmentation
60 model to different channel configurations, which can be impractical.

61 To overcome this limitation, a hybrid workflow combining unsupervised and supervised learning
62 methods for cell segmentation was proposed [18]. This hybrid workflow uses Ilastik [27], an
63 interactive image processing tool, to generate a probability map based on multiple rounds of user
64 inputs and adjustments. In each round, a user only needs to perform a limited number of annotations
65 on regions where the probability map generated based on previous annotations is not satisfactory.
66 CellProfiler is then used to perform the single cell segmentation based on the probability map once
67 the result from Ilastik is acceptable. This hybrid workflow significantly reduces manual annotation
68 workload and has gained popularity in many recent IMC studies [10, 13, 14, 17, 21]. However, the
69 annotation process still needs to be performed by experienced staff repeatedly for each IMC study,
70 which is very inconvenient. In addition, the reproducibility of the experimental results obtained
71 from this approach can be an issue due to the per-study, interactive training process used in creating
72 the single cell masks. Hence, a more efficient, fully automated single-cell segmentation method for
73 IMC data without compromising the segmentation accuracy is necessary for IMC to gain broader
74 applications in biomedical studies.

75 Convolutional neural networks (CNNs) have been successfully used for natural image segmen-
76 tation and recently applied in biomedical image applications [29, 30, 31, 32]. CNN-based U-Net
77 was developed for pixel-wise cell segmentation of mammalian cells [33]. It has been demonstrated
78 that the U-Net architecture and its variants such as Unet++[34], 3D Unet [35] and V-Net [36] can
79 obtain high segmentation accuracy. Motivated by the outstanding performance of using U-Nets
80 for cell segmentation [37, 38, 39], we developed Dice-XMBD, a deep neural network (DNN)-based
81 cell segmentation method for multichannel IMC images. Dice-XMBD is marker agnostic and can
82 perform cell segmentation for IMC images of different channel configurations without modification.
83 To achieve this goal, Dice-XMBD first merges multiple-channel IMC images into two channels
84 consisting of a nuclear channel containing proteins originated from cell nucleus, and a cell channel
85 containing proteins originated from cytoplasm and cell membrane. Channels of proteins with

86 ambiguous locations are ignored by Dice-XMBD for segmentation as they contribute little to the
87 segmentation results. Furthermore, to mitigate the annotation workload, we adopted the knowledge
88 distillation learning framework [40] in training Dice-XMBD, where the training labels were generated
89 using Ilastik with interactive manual annotation as a teacher model. We used four IMC datasets
90 of different channel configurations to evaluate the performance of Dice-XMBD and the results
91 show that it can generate highly accurate cell segmentation results that are comparable to those
92 from manual annotation for IMC images from both the same and different datasets to the training
93 dataset, validating its applicability for generic IMC image segmentation tasks.

94 **2 Materials and methods**

95 **2.1 Overview of the pipeline**

96 In Dice-XMBD, we used a U-Net based pixel classification model to classify individual pixels of
97 an IMC image to their cellular origins, namely, nuclei, cytoplasm/membrane, or background. The
98 pixel probability map produced by the classifier was then used by CellProfiler (version 3.1.0) to
99 produce the final cell segmentation results (Figure 1). The pixel classification model was trained on
100 IMC images with pixel-level annotations. To mitigate the annotation workload, Ilastik was used as
101 the teacher model to produce the classification labels for training. Furthermore, to obtain a generic
102 pixel classifier that can be used across IMC datasets of different channel configuration, channels of
103 different proteins were combined based on their cellular origins into two channels, namely, nuclear
104 and cell (membrane/cytoplasmic) channels, respectively. Channels of proteins without specific
105 cellular locations were ignored by Dice-XMBD. The pixel classification model was then trained on
106 the combined two-channel images. Likewise, the same preprocessing was used at the prediction
107 stage to produce the two-channel (nuclear/cell) images for pixel classification. Of note, although the
108 prediction may be performed on images with different markers, the channels were always combined
109 based on their origins so that pixel classification was performed based on the two channels of
110 putative protein locations rather than channels of individual proteins.

111 **2.2 Training and evaluation datasets**

112 We used four IMC image datasets in this study. BRCA1 and BRCA2 [18] contain 548 and 746
113 images from patients with breast cancer with 37 and 35 markers respectively. T1D1 [10] and T1D2
114 [11] contain 839 and 754 images from patients with Type I Diabetes with 35 and 33 markers,
115 respectively. Dice-XMBD was trained on a subset of BRCA1 dataset ($n = 348$) with 200 held-out
116 images reserved for validation and test. To test the generalization ability of Dice-XMBD, we also
117 tested the trained model on the other three independent IMC datasets (BRCA2, T1D1 and T1D2).

118 **2.3 Generating groundtruth cell masks**

119 The groundtruth cell masks for training were generated using Ilastik and CellProfiler. We used the
120 smallest brush size (1 pixel) in annotating the image to avoid annotating a group of neighboring
121 pixels of different classes. The annotation was performed in an interactive manner, where the
122 random forest prediction model of Ilastik was updated regularly during annotation to produce
123 an uncertainty map indicating the confidence level of the classification results produced by the
124 prediction model. The annotation was then guided by the uncertainty map to focus on the regions
125 with high uncertainty iteratively until the overall uncertainty values were low except for regions of
126 which the boundaries were visually indistinguishable.

127 The initial annotation was performed on a randomly selected subset of the dataset. After
128 the initial annotation, we loaded all the images from the dataset into Ilastik to calculate their
129 uncertainty maps, and then selected those with the highest average uncertainty values for further
130 annotation. This process was iterated until the uncertainty values of all images converged, i.e., did
131 not significantly decrease for three iterations.

132 In the end, we annotated 49 images in BRCA1 to train the model in Ilastik. We then imported
133 all the images of the BRCA1 dataset into Ilastik for batch processing and export their corresponding
134 pixel classification probability maps for training Dice-XMBD. The probability maps were further
135 input to CellProfiler to produce the “groundtruth” cell segmentation. In CellProfiler, we used the
136 “IdentifyPrimaryObjects” module to segment the cell nuclei and used the “IdentifySecondaryObjects”
137 to segment the cell membranes using the propagation method. The output masks from CellProfiler
138 are regarded as “groundtruth” cell segmentation of the dataset for performance evaluation.

139 We also generated the groundtruth cell masks of the other three datasets by the same iterative
140 procedure separately for testing the generalization ability of Dice-XMBD. During the process, 72
141 images in BRCA2, 39 images in T1D1, and 67 images in T1D2 were manually annotated.

142 2.4 Training the U-Net cell segmentation model

143 2.4.1 Image preprocessing

144 The input IMC images were firstly preprocessed by hot pixel removal, dynamic range conversion,
145 normalization, and image cropping/padding into fix-sized patches. First, we applied a 5×5 low-pass
146 filter on the image to remove hot pixels. If the difference between an image pixel value and the
147 corresponding filtered value was larger than a preset threshold (50 in our experiments), the pixel
148 would be regarded as a hot pixel and its value would be replaced by the filtered value. As the
149 dynamic range of pixels values differs among IMC images of different batches and different channels,
150 we further min-max normalized all images to $[0,255]$ to remove such batch effect as follows:

$$x'_{ij} = \frac{x_{ij} - X_{min}}{X_{max} - X_{min}} * 255, \quad (1)$$

151 where x_{ij} is the pixel value in one channel, X_{max} and X_{min} denote the maximum and minimum
152 values in the channel. Of note, as the pixel values in IMC images have a high dynamic range,
153 transforming the pixel values from its dynamic range to $[0, 255]$ would suffer from detail suppression
154 by one or few extremely large values. Therefore, we thresholded the image pixel values at 99.7%
155 percentile for each image before normalization.

156 Finally, we merged all the nuclear channels into the nuclear channel, and membrane/cytoplasmic
157 channels into the cell channel, by averaging on all channel images with pre-selected sets of protein
158 markers, respectively. We converted the merged two-channel images into patches of 512×512 pixels.
159 For images or boundary patches that are small than target patch size, we set the pixel values of
160 both channels to 0 and set the pixel type as background for padding.

161 2.4.2 Data augmentation

162 Data augmentation is an efficient strategy to reduce overfitting and enhance the robustness of
163 the trained models especially when training data is insufficient. We applied the following data
164 augmentation methods on input images.

165 First, photometric transformations including contrast stretching and intensity adjustments were
166 used. For contrast stretching, we changed the level of contrast by multiplication with a random
167 factor in the range of $[0.5, 1.5]$. Similarly, for intensity adjustments we changed the level of intensity

168 by multiplication with a random factor in the range of [0.5, 1.5]. Geometric transformations
169 including image flipping and rotation were used. For flipping we implemented random horizontal
170 or vertical flipping. For rotation, the rotating angle is randomly distributed in the range of [-180,
171 180]. Note that geometric transformations were applied to pairs of input and output images of the
172 network. We also injected random Gaussian noise to the two input channels of the input images.
173 Examples of data augmentation are shown in Supplementary Figures 1 and 2.

174 2.4.3 Constructing a pixel classification model

175 The U-Net pixel classification network is an end-to-end fully convolutional network and contains
176 two paths. The contracting path (or the encoder) uses a typical CNN architecture. Each block
177 in the contracting path consists of two successive 3×3 convolution layers followed by a Rectified
178 Linear Unit (ReLU) activation and a 2×2 max-pooling layer. This block is repeated four times. In
179 the symmetric expansive path (or the decoder), at each stage the feature map is upsampled using
180 2×2 up-convolution. To enable precise localization, the feature map from the corresponding layer
181 in the contracting path is cropped and concatenated onto the upsampled feature map, followed
182 by two successive 3×3 convolutions and ReLU activation. At the final stage, an additional 1×1
183 convolution is applied to reduce the feature map to the required number of channels. Three channels
184 are used in our case for cell nuclei, membrane, and background, respectively. As we output the
185 probability map, the output values are converted into the range of [0, 1] using the Sigmoid function.

186 2.4.4 Loss function

187 We take the binary cross-entropy (BCE) as the loss function which is defined as:

$$188 \text{loss}(y, \hat{y}) = -\frac{1}{N} \sum_{i=0}^N (y_i * \log(\hat{y}_i) + (1 - y_i) * \log(1 - \hat{y}_i)), \quad (2)$$

189 where N represents the total number of pixels in an image, y_i denotes the ground truth pixel
190 probability and \hat{y}_i denotes the predicted pixel probability. The cross-entropy loss compares the
191 predicted probabilities with the ground truth values. The loss is minimized during the training
192 process.

192 2.5 Model evaluation

193 To evaluate pixel-level accuracy, we calculated the true positive and false positive based on the
194 binary cell masks. In a binary cell mask, 1 represents cell boundary and 0 denotes cell interior or
195 exterior. For every pixel in an image, true positive (TP) and true negative (TN) mean that the
196 predicted pixel classification is the same with its label, while false positive (FP) and false negative
197 (FN) mean that a pixel is misclassified as cell boundary or cell interior/exterior, respectively. To
198 evaluate model performance at cell-level, we first calculated the intersection over union (IOU) on
199 cells from predicted and labeled cell masks to determine if they are the same cell from different cell
200 segmentation. After filtering cell matches with IOU below 0.1, if a predicted cell only finds one true
201 cell, the cell is segmented accurately (same as TP). If a true cell cannot find a predicted cell, the
202 cell is denoted as FN. Also, there exist some predicted cells which are assigned to the same true cell,
203 we consider this situation as a split error. If multiple true cells are matched to a same predicted cell,
204 we consider those predicted cells as merge errors. Four standard indices are measured as below.

$$205 \text{Recall} = \frac{TP}{TP + FN}, \quad (3)$$

$$Precision = \frac{TP}{TP + FP}, \quad (4)$$

$$F1score = \frac{2 * Precision * Recall}{Precision + Recall}, \quad (5)$$

$$Jaccard = \frac{TP}{TP + FP + FN}. \quad (6)$$

205 3 RESULTS

206 3.1 Dice-XMBD enables automatic cell segmentation

207 We trained a deep learning model with the above-described U-Net architecture using the BRCA1
208 dataset with 348 images as training set and 100 images as validation set. A complete held out test
209 set with 100 images was used to test model performance within one dataset. We further applied
210 the trained model directly on the other three IMC image datasets to evaluate the cross-dataset
211 performance of the model. To evaluate the model performance, we computed standard indices
212 (Recall, Precision, F1-score, and Jaccard index) for both pixel-level and cell-level accuracies (see
213 Methods for more details).

214 We compared Dice-XMBD with a generic whole-cell segmentation method across six imaging
215 platforms, Mesmer [41], which used a deep learning-based algorithm trained on a large, annotated
216 image dataset to segment single cells and nuclei separately. A trained Mesmer model was tested
217 with combined nuclear and cell channels which is the same as the input to Dice-XMBD. Meanwhile,
218 we used three commonly used segmentation methods implemented in CellProfiler with default
219 parameters: distance, watershed, and propagation. These methods first find nuclei as primary
220 objects, and then the membrane proteins are added together into an image as input to recognize
221 cells. The distance method does not use any membrane proteins information and simply defines cell
222 membrane by expanding several pixels around nuclei. The watershed method computes intensity
223 gradients on the Sobel transformed image to find boundary between cells [42], while the propagation
224 method defines cell boundary by combining the distance of the nearest primary object and intensity
225 gradients of cell membrane image [43]. Hereafter we refer these methods as CP-distance, CP-
226 watershed, and CP-propagation, respectively. Results show that Dice-XMBD outperformed all
227 other benchmarked methods with highest accuracy on pixel level (F1 score = 0.92, Jaccard index
228 = 0.85) (Figure 2A). We also observed that CP-distance obtained the highest recall (Recall =
229 0.95) but lowest precision (Precision = 0.66), which means that it can identify almost every pixel
230 correctly in the labeled mask but only 66% of predicted pixels were accurate.

231 In terms of cell-level performance, we first counted cells per image from predicted and labeled
232 cell masks. Dice-XMBD can predict cells similar to ground truth (Pearson correlation = 0.998).
233 Although the correlation between prediction and ground truth was relatively high among all
234 segmentation methods, Mesmer tended to predict less cells while CellProfiler was more likely to
235 over-split cells, as shown in Figure 2B and Figure 2C. Moreover, Figure 2C shows that Dice-XMBD
236 had the best prediction performance (F1-score = 0.856) considering precision (Precision = 0.880,
237 percent of cells that were correctly predicted) and recall (Recall = 0.867, percent of true cells that
238 are predicted) than Mesmer (F1-score = 0.557) and CellProfiler (F1-score \approx 0.56). We further
239 checked the IOU distribution of all one-to-one cell pairs (predicted and true cells), Figure 2D
240 demonstrates that most matched cell pairs predicted from Dice-XMBD were highly overlapping
241 (mean = 0.815, median = 0.821), followed by Mesmer where most matched pairs are only half area of

242 overlap (mean = 0.579, median = 0.595). An example of BRCA1 shown in Figure 2E demonstrates
243 Dice-XMBD prediction was far superior to other benchmarked methods since it contained most
244 cells with high matched values.

245 **3.2 Dice-XMBD enables generic IMC image segmentation tasks**

246 The key idea of this study was to generate an IMC-specific single cell segmentation model across
247 different datasets with multiple proteins. We selected three independent IMC datasets generated
248 from different labs to test the generalization ability of Dice-XMBD. Apart from the benchmarked
249 methods mentioned above, we also included Ilastik model trained from BRCA1 annotations in
250 our comparison. Figure 3A shows that Dice-XMBD outperformed all the methods, followed by
251 Ilastik. Moreover, the performance of cells prediction from Dice-XMBD was the best and the most
252 stabilized among three datasets, while Ilastik and Mesmer tended to under-predict cells. CellProfiler
253 predicted less cells in BRCA2 and over-predicted cells in two T1D datasets, as shown in Figure 3B
254 and Figure 3C. Furthermore, Dice-XMBD predictions contained most of the cells with IOU value
255 higher than 0.8 (Figure 3D and Supplementary Figure S3).

256 **3.3 Dice-XMBD enables accurate downstream biological analysis**

257 Single cell segmentation is the first step of downstream analysis, such as protein quantification, cells
258 clustering and annotation. Figure 4A shows the distribution of five proteins extracted from one
259 image of BRCA1, proteins distribution from Dice-XMBD are more similar to them from ground
260 truth than other methods. We calculated Pearson correlation of protein profiling between prediction
261 and ground truth, which demonstrated that protein profiling correlation from Dice-XMBD was the
262 highest among all methods by testing in the same dataset (BRCA1) and across datasets (BRCA2,
263 T1D1, T1D2), as shown in Figures 4B and 4C. These results suggest that Dice-XMBD has good
264 generalization ability to predict single cells for different IMC images with minimum impact to the
265 downstream analysis due to the high correlation of its results with ground truth.

266 **4 DISCUSSION**

267 Highly multiplexed single cell imaging technologies such as IMC are becoming increasingly important
268 tools for both basic biomedical and clinical research. These tools can unveil complex single-cell
269 phenotypes and their spatial context at unprecedented details, which provide a solid base for further
270 exploration in cancer, diabetes, and other complex diseases. Nevertheless, cell segmentation has
271 become a major bottleneck in analyzing multiplexed images. Conventional approaches rely on
272 intensities of protein markers to identify different cellular structures such as nuclei, cytoplasm,
273 and membrane. Unfortunately, the intensity values of these markers are strongly cell type-specific
274 and may vary from cells to cells. In addition, the staining also shows variability across images or
275 datasets. As a result, the accuracy and robustness of the segmentation results are far from optimal.
276 On the other hand, high-order visual features including spatial distribution of markers, textures,
277 and gradients are relevant to visually identify subcellular structures by human. However, those
278 features are not considered in these methods to improve the cell segmentation results.

279 The DNN-based image segmentation approaches provide an opportunity to leverage high-order
280 visual features at cellular level for better segmentation results. Unfortunately, they require a
281 significant amount of annotation data that are in general difficult to acquire. In addition, the
282 highly variable channel configurations of multiplexed images impose another important obstacle
283 to the usability of these methods as most of them lack the ability to adapt to different channel

284 configurations after models are trained. In this study, we develop Dice-XMBD, a generic solution for
285 IMC image segmentation based on U-Net. Dice-XMBD overcomes the limitation of training data
286 scarcity and achieves human-level accuracy by distilling expert knowledge from Ilastik with manual
287 input of human as a teacher model. Moreover, by consolidating multiple channels of different
288 proteins into two cellular structure aware channels, Dice-XMBD provides an effective off-the-shelf
289 solution for cell segmentation tasks across different studies without retraining that can lead to
290 significant delay in analysis. Finally, to facilitate the analysis of large amount of IMC data currently
291 being generated around the world, we made Dice-XMBD publicly available as an open-source
292 software on GitHub (<https://github.com/xmuyulab/Dice-XMBD>).

293 Conflict of Interest Statement

294 RY and WY are shareholders of Aginome Scientific. The authors declare no other conflict of
295 interest.

296 Author Contributions

297 WY, LW, RY and JH discussed the ideas and supervised the study. YQ and YJ implemented and
298 conducted experiments in deep network cell segmentation. XX performed the model evaluation and
299 biological analysis on segmentation results. XX, WY and RY wrote the manuscript. All authors
300 discussed and commented on the manuscript.

301 Data Availability Statement

302 All datasets used for this study can be found at GitHub (<https://github.com/xmuyulab/Dice-XMBD>).
303 These datasets are downloaded from: BRCA1 ([https://idr.openmicroscopy.org/search/?query=](https://idr.openmicroscopy.org/search/?query=Name:idr0076-ali-metabric/experimentA)
304 [Name:idr0076-ali-metabric/experimentA](https://idr.openmicroscopy.org/search/?query=Name:idr0076-ali-metabric/experimentA)), BRCA2 ([https://zenodo.org/record/3518284#](https://zenodo.org/record/3518284#.YLnm1S8RquU)
305 [.YLnm1S8RquU](https://zenodo.org/record/3518284#.YLnm1S8RquU)), T1D1 (<https://data.mendeley.com/datasets/cydmwsfztj/1>), T1D2 (part1:
306 <https://data.mendeley.com/datasets/9b262xmtm9/1>, part2: [https://data.mendeley.com/datasets/
307 \[xbxnfg2zfs/1\]\(https://data.mendeley.com/datasets/xbxnfg2zfs/1\)](https://data.mendeley.com/datasets/xbxnfg2zfs/1)), respectively.

308 References

- 309 [1] D. Lähnemann, J. Köster, E. Szczurek, D. J. McCarthy, S. C. Hicks, M. D. Robinson, C. A.
310 Vallejos, K. R. Campbell, N. Beerenwinkel, A. Mahfouz, *et al.*, “Eleven grand challenges in
311 single-cell data science,” *Genome biology*, vol. 21, no. 1, pp. 1–35, 2020.
- 312 [2] M. J. Stubbington, O. Rozenblatt-Rosen, A. Regev, and S. A. Teichmann, “Single-cell tran-
313 scriptomics to explore the immune system in health and disease,” *Science*, vol. 358, no. 6359,
314 pp. 58–63, 2017.
- 315 [3] S. S. Potter, “Single-cell rna sequencing for the study of development, physiology and disease,”
316 *Nature Reviews Nephrology*, vol. 14, no. 8, pp. 479–492, 2018.
- 317 [4] E. Papalexi and R. Satija, “Single-cell rna sequencing to explore immune cell heterogeneity,”
318 *Nature Reviews Immunology*, vol. 18, no. 1, p. 35, 2018.

- 319 [5] W. C. C. Tan, S. N. Nerurkar, H. Y. Cai, H. H. M. Ng, D. Wu, Y. T. F. Wee, J. C. T. Lim,
320 J. Yeong, and T. K. H. Lim, “Overview of multiplex immunohistochemistry/immunofluorescence
321 techniques in the era of cancer immunotherapy,” *Cancer Communications*, vol. 40, no. 4,
322 pp. 135–153, 2020.
- 323 [6] C. Giesen, H. A. Wang, D. Schapiro, N. Zivanovic, A. Jacobs, B. Hattendorf, P. J. Schüffler,
324 D. Grolimund, J. M. Buhmann, S. Brandt, *et al.*, “Highly multiplexed imaging of tumor tissues
325 with subcellular resolution by mass cytometry,” *Nature methods*, vol. 11, no. 4, pp. 417–422,
326 2014.
- 327 [7] P. Zrazhevskiy and X. Gao, “Quantum dot imaging platform for single-cell molecular profiling,”
328 *Nature communications*, vol. 4, no. 1, pp. 1–12, 2013.
- 329 [8] M. Angelo, S. C. Bendall, R. Finck, M. B. Hale, C. Hitzman, A. D. Borowsky, R. M. Levenson,
330 J. B. Lowe, S. D. Liu, S. Zhao, *et al.*, “Multiplexed ion beam imaging of human breast tumors,”
331 *Nature medicine*, vol. 20, no. 4, pp. 436–442, 2014.
- 332 [9] Q. Chang, O. I. Ornatsky, I. Siddiqui, A. Loboda, V. I. Baranov, and D. W. Hedley, “Imaging
333 mass cytometry,” *Cytometry part A*, vol. 91, no. 2, pp. 160–169, 2017.
- 334 [10] N. Damond, S. Engler, V. R. Zanotelli, D. Schapiro, C. H. Wasserfall, I. Kusmartseva, H. S.
335 Nick, F. Thorel, P. L. Herrera, M. A. Atkinson, *et al.*, “A map of human type 1 diabetes
336 progression by imaging mass cytometry,” *Cell metabolism*, vol. 29, no. 3, pp. 755–768, 2019.
- 337 [11] Y. J. Wang, D. Traum, J. Schug, L. Gao, C. Liu, M. A. Atkinson, A. C. Powers, M. D. Feldman,
338 A. Naji, K.-M. Chang, *et al.*, “Multiplexed in situ imaging mass cytometry analysis of the
339 human endocrine pancreas and immune system in type 1 diabetes,” *Cell metabolism*, vol. 29,
340 no. 3, pp. 769–783, 2019.
- 341 [12] V. Ramaglia, S. Sheikh-Mohamed, K. Legg, C. Park, O. L. Rojas, S. Zandee, F. Fu, O. Ornatsky,
342 E. C. Swanson, D. Pitt, *et al.*, “Multiplexed imaging of immune cells in staged multiple sclerosis
343 lesions by mass cytometry,” *Elife*, vol. 8, p. e48051, 2019.
- 344 [13] C. Böttcher, M. van der Poel, C. Fernández-Zapata, S. Schlickeiser, J. K. Leman, C.-C. Hsiao,
345 M. R. Mizee, M. C. Vincenten, D. Kunkel, I. Huitinga, *et al.*, “Single-cell mass cytometry
346 reveals complex myeloid cell composition in active lesions of progressive multiple sclerosis,”
347 *Acta neuropathologica communications*, vol. 8, no. 1, pp. 1–18, 2020.
- 348 [14] N. L. de Vries, A. Mahfouz, F. Koning, and N. F. de Miranda, “Unraveling the complexity
349 of the cancer microenvironment with multidimensional genomic and cytometric technologies,”
350 *Frontiers in Oncology*, vol. 10, p. 1254, 2020.
- 351 [15] S. Brähler, B. H. Zinselmeyer, S. Raju, M. Nitschke, H. Suleiman, B. T. Saunders, M. W.
352 Johnson, A. M. Böhner, S. F. Viehmann, D. J. Theisen, *et al.*, “Opposing roles of dendritic
353 cell subsets in experimental gn,” *Journal of the American Society of Nephrology*, vol. 29, no. 1,
354 pp. 138–154, 2018.
- 355 [16] T. Aoki, L. C. Chong, K. Takata, K. Milne, M. Hav, A. Colombo, E. A. Chavez, M. Nissen,
356 X. Wang, T. Miyata-Takata, *et al.*, “Single-cell transcriptome analysis reveals disease-defining
357 t-cell subsets in the tumor microenvironment of classic hodgkin lymphoma,” *Cancer Discovery*,
358 vol. 10, no. 3, pp. 406–421, 2020.

- 359 [17] H. W. Jackson, J. R. Fischer, V. R. Zanutelli, H. R. Ali, R. Mechera, S. D. Soysal, H. Moch,
360 S. Muenst, Z. Varga, W. P. Weber, *et al.*, “The single-cell pathology landscape of breast cancer,”
361 *Nature*, vol. 578, no. 7796, pp. 615–620, 2020.
- 362 [18] H. R. Ali, H. W. Jackson, V. R. T. Zanutelli, E. Danenberg, J. R. Fischer, H. Bardwell,
363 E. Provenzano, O. M. Rueda, S.-F. Chin, S. Aparicio, C. Caldas, and B. Bodenmiller, “Imaging
364 mass cytometry and multiplatform genomics define the phenogenomic landscape of breast
365 cancer,” *Nature Cancer*, vol. 1, no. 2, pp. 163–175, 2020.
- 366 [19] P. Dey, J. Li, J. Zhang, S. Chaurasiya, A. Strom, H. Wang, W.-T. Liao, F. Cavallaro, P. Denz,
367 V. Bernard, *et al.*, “Oncogenic kras-driven metabolic reprogramming in pancreatic cancer
368 cells utilizes cytokines from the tumor microenvironment,” *Cancer discovery*, vol. 10, no. 4,
369 pp. 608–625, 2020.
- 370 [20] Y. Zhang, Y. Gao, L. Qiao, W. Wang, and D. Chen, “Inflammatory response cells during acute
371 respiratory distress syndrome in patients with coronavirus disease 2019 (covid-19),” *Annals of*
372 *Internal Medicine*, 2020.
- 373 [21] B. Bengsch, M. Schwabenland, H. Salié, J. Tanevski, S. Killmer, J. Matschke, K. Püschel,
374 H. Mei, T. Boettler, C. Neumann-Haefelin, *et al.*, “Deep spatial profiling of covid19 brains
375 reveals neuroinflammation by compartmentalized local immune cell interactions and targets
376 for intervention,” 2020.
- 377 [22] D. Schulz, V. R. T. Zanutelli, J. R. Fischer, D. Schapiro, S. Engler, X.-K. Lun, H. W. Jackson,
378 and B. Bodenmiller, “Simultaneous multiplexed imaging of mrna and proteins with subcellular
379 resolution in breast cancer tissue samples by mass cytometry,” *Cell systems*, vol. 6, no. 1,
380 pp. 25–36, 2018.
- 381 [23] L. E. Flint, G. Hamm, J. D. Ready, S. Ling, C. J. Duckett, N. A. Cross, L. M. Cole, D. P.
382 Smith, R. J. Goodwin, and M. R. Clench, “Characterization of an aggregated three-dimensional
383 cell culture model by multimodal mass spectrometry imaging,” *Analytical Chemistry*, vol. 92,
384 no. 18, pp. 12538–12547, 2020.
- 385 [24] R. Catena, A. Oezcan, L. Kuett, A. Pluess, P. Schraml, H. Moch, B. Bodenmiller, I. Consortium,
386 *et al.*, “Highly multiplexed molecular and cellular mapping of breast cancer tissue in three
387 dimensions using mass tomography,” *bioRxiv*, 2020.
- 388 [25] A. Bouzekri, A. Esch, and O. Ornatsky, “Multidimensional profiling of drug-treated cells by
389 imaging mass cytometry,” *FEBS open bio*, vol. 9, no. 9, pp. 1652–1669, 2019.
- 390 [26] A. E. Carpenter, T. R. Jones, M. R. Lamprecht, C. Clarke, I. H. Kang, O. Friman, D. A.
391 Guertin, J. H. Chang, R. A. Lindquist, J. Moffat, *et al.*, “Cellprofiler: image analysis software
392 for identifying and quantifying cell phenotypes,” *Genome biology*, vol. 7, no. 10, p. R100, 2006.
- 393 [27] C. Sommer, C. Straehle, U. Köthe, and F. A. Hamprecht, “Ilastik: Interactive learning and
394 segmentation toolkit,” in *2011 IEEE International Symposium on Biomedical Imaging: From*
395 *Nano to Macro*, pp. 230–233, 2011.
- 396 [28] X. Liu, W. Song, B. Y. Wong, T. Zhang, S. Yu, and G. N. Lin, “A comparison framework and
397 guideline of clustering methods for mass cytometry data,” *Genome Biology*, vol. 20, no. 297,
398 pp. 1–18, 2019.

- 399 [29] D. Shen, G. Wu, and H.-I. Suk, “Deep learning in medical image analysis,” *Annual review of*
400 *biomedical engineering*, vol. 19, pp. 221–248, 2017.
- 401 [30] M. Zhang, X. Li, M. Xu, and Q. Li, “Rbc semantic segmentation for sickle cell disease
402 based on deformable u-net,” in *International Conference on Medical Image Computing and*
403 *Computer-Assisted Intervention*, pp. 695–702, Springer, 2018.
- 404 [31] A. R. Andrade, L. H. Vogado, R. de MS Veras, R. R. Silva, F. H. Araujo, and F. N. Medeiros,
405 “Recent computational methods for white blood cell nuclei segmentation: A comparative study,”
406 *Computer Methods and Programs in Biomedicine*, vol. 173, pp. 1–14, 2019.
- 407 [32] T. Vicar, J. Balvan, J. Jaros, F. Jug, R. Kolar, M. Masarik, and J. Gumulec, “Cell segmentation
408 methods for label-free contrast microscopy: review and comprehensive comparison,” *BMC*
409 *bioinformatics*, vol. 20, no. 1, p. 360, 2019.
- 410 [33] O. Ronneberger, P. Fischer, and T. Brox, “U-net: Convolutional networks for biomedical image
411 segmentation,” in *International Conference on Medical image computing and computer-assisted*
412 *intervention*, pp. 234–241, Springer, 2015.
- 413 [34] Z. Zhou, M. M. R. Siddiquee, N. Tajbakhsh, and J. Liang, “Unet++: A nested u-net
414 architecture for medical image segmentation,” in *Deep Learning in Medical Image Analysis*
415 *and Multimodal Learning for Clinical Decision Support*, pp. 3–11, Springer, 2018.
- 416 [35] Ö. Çiçek, A. Abdulkadir, S. S. Lienkamp, T. Brox, and O. Ronneberger, “3d u-net: learning
417 dense volumetric segmentation from sparse annotation,” in *International conference on medical*
418 *image computing and computer-assisted intervention*, pp. 424–432, Springer, 2016.
- 419 [36] F. Milletari, N. Navab, and S.-A. Ahmadi, “V-net: Fully convolutional neural networks for
420 volumetric medical image segmentation,” in *2016 fourth international conference on 3D vision*
421 *(3DV)*, pp. 565–571, IEEE, 2016.
- 422 [37] D. A. Van Valen, T. Kudo, K. M. Lane, D. N. Macklin, N. T. Quach, M. M. DeFelice, I. Maayan,
423 Y. Tanouchi, E. A. Ashley, and M. W. Covert, “Deep learning automates the quantitative
424 analysis of individual cells in live-cell imaging experiments,” *PLoS computational biology*,
425 vol. 12, no. 11, p. e1005177, 2016.
- 426 [38] D. Salem, Y. Li, P. Xi, M. Cuperlovic-Culf, H. Phenix, and M. Kaern, “Yeastnet: Deep learning
427 enabled accurate segmentation of budding yeast cells in bright-field microscopy,” *bioRxiv*, 2020.
- 428 [39] R. Hollandi, A. Szkalitsy, T. Toth, E. Tasnadi, C. Molnar, B. Mathe, I. Grexa, J. Molnar,
429 A. Balind, M. Gorbe, *et al.*, “nucleaizer: A parameter-free deep learning framework for nucleus
430 segmentation using image style transfer,” *Cell Systems*, 2020.
- 431 [40] G. Hinton, O. Vinyals, and J. Dean, “Distilling the knowledge in a neural network,” in *NIPS*
432 *Deep Learning and Representation Learning Workshop*, 2015.
- 433 [41] N. F. Greenwald, G. Miller, E. Moen, A. Kong, A. Kagel, C. C. Fullaway, B. J. McIntosh,
434 K. Leow, M. S. Schwartz, T. Dougherty, *et al.*, “Whole-cell segmentation of tissue images with
435 human-level performance using large-scale data annotation and deep learning,” *bioRxiv*, 2021.
- 436 [42] L. Vincent and P. Soille, “Watersheds in digital spaces: an efficient algorithm based on
437 immersion simulations,” *IEEE Computer Architecture Letters*, vol. 13, no. 06, pp. 583–598,
438 1991.

439 [43] T. R. Jones, A. Carpenter, and P. Golland, "Voronoi-based segmentation of cells on image
440 manifolds," in *International Workshop on Computer Vision for Biomedical Image Applications*,
441 pp. 535–543, Springer, 2005.

442 Figure captions

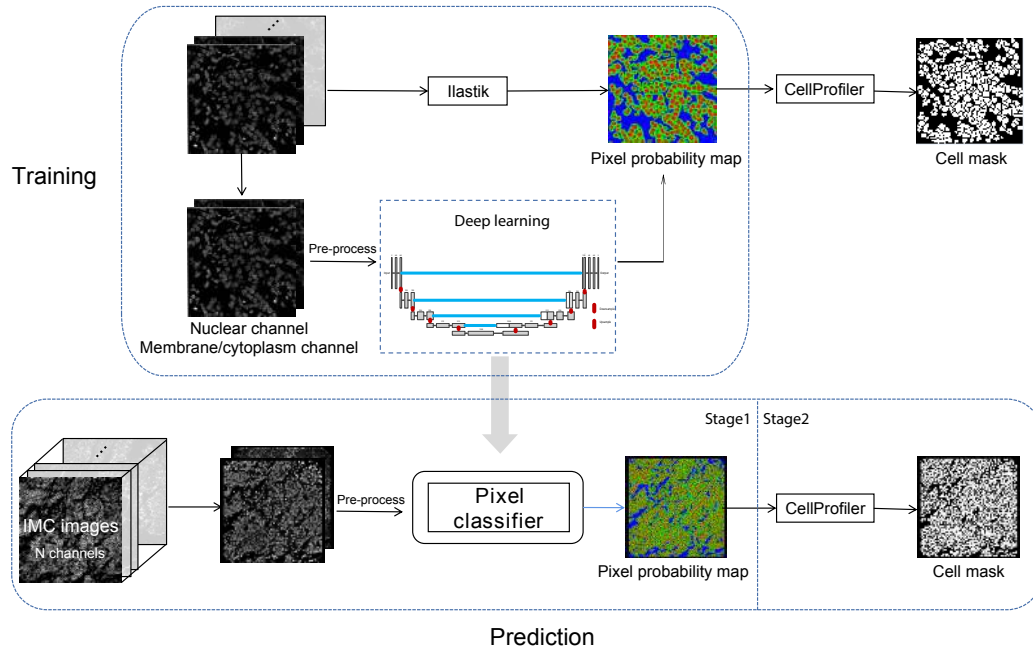


Figure 1: Dice-XMBD workflow. IMC images are combined into 2-channel images containing membrane/cytoplasm and nuclear proteins expression information. In stage 1, the pixel probability maps of given 2-channel images are predicted using a semi-supervised learning model based on Unet architecture. The training data were generated from Ilastik by using human annotations. In stage 2, the cell segmentation masks are generated from the pixel probability maps using the propagation method in CellProfiler.

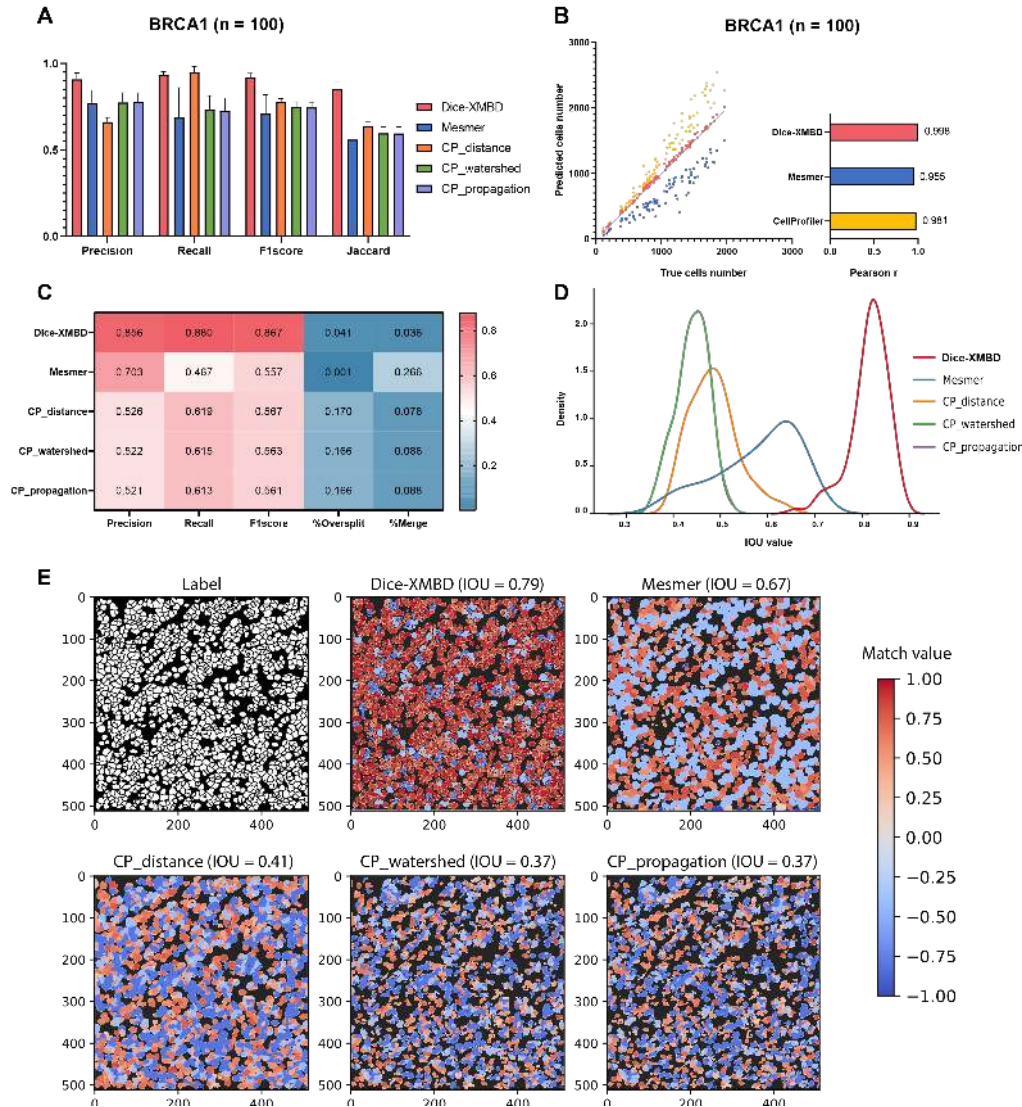


Figure 2: Dice-XMBD enables automatic single cell segmentation on dataset BRCA1. **(A)** Pixel prediction performance comparisons of Dice-XMBD, Mesmer and CellProfiler (CP-distance, CP-watershed, CP-propagation). All data in bar plots are presented as mean values \pm SD. **(B)** Pearson correlations between the number of predicted cells and labelled cells per image. The number of cells predicted from three cell segmentation methods implemented in CellProfiler are the same (represented as CellProfiler). **(C)** Cell prediction performance of five benchmarked methods. The percent of oversplits and merge errors in predictions are denoted as %Oversplit and %Merge. **(D)** Density plot shows the distribution of mean IOU values of matched cells per image. **(E)** An example of labeled and predicted single cell mask from benchmarked methods. Match value represents IOU value for one-to-one cell pair found in label and prediction, -0.4 and -0.8 for merged cells (multiple true cells are assigned to one predicted cell) and split cells (multiple predicted cells are matched to a true cell), and -1 for cells are not in any of above situations. Numbers in brackets of each method indicate mean of IOU values of all matched cell pairs.

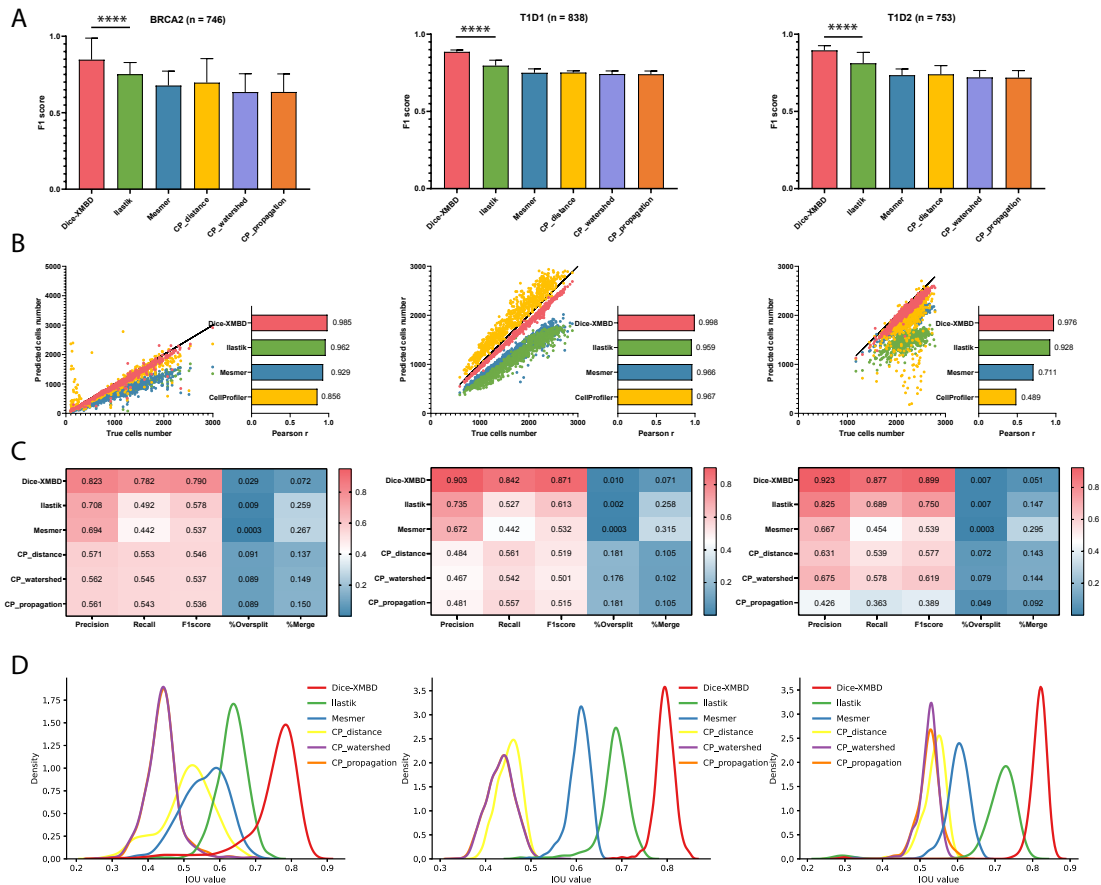


Figure 3: Dice-XMBD enables generic IMC image segmentation. Left: BRCA2, middle: T1D1, right: T1D2. **(A)** Pixel prediction performance comparisons of Dice-XMBD, Ilastik, Mesmer and CellProfiler (CP-distance, CP-watershed, CP-propagation). All data in bar plots are presented as mean values +/- SD. **(B)** Pearson correlations between the number of predicted cells and labelled cells per image. The number of cells predicted from three cell segmentation methods implemented in CellProfiler are the same (represented as CellProfiler). **(C)** Heatmaps of cells prediction performance of six benchmarked methods. The percent of oversplit and merge errors in predictions are denoted as %Oversplit and %Merge. **(D)** Density plots show the distribution of mean IOU values of matched cells per image.

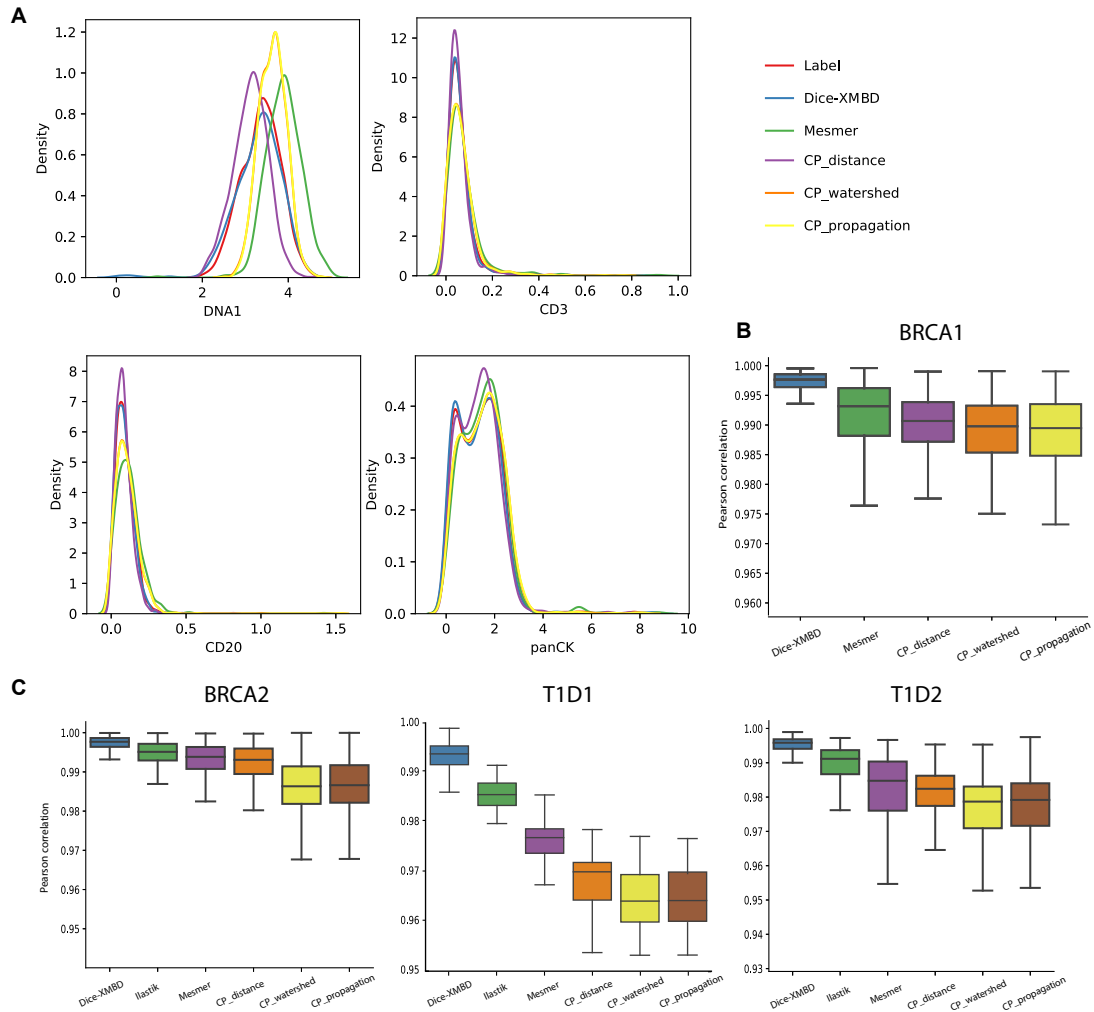


Figure 4: Dice-XMBD enables accurate downstream biological analysis. **(A)** Four protein distribution of one image of BRCA1. Sing cell protein profiling Pearson correlation between prediction and ground truth for BRCA1 dataset **(B)** and other three datasets **(C)**. Boxplots represent median with the center line, and whiskers extend to 1.5x interquartile range (IQR).