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Supplemental information

DeepSea is an efficient deep-learning model for single-cell segmentation and tracking in time-lapse microscopy

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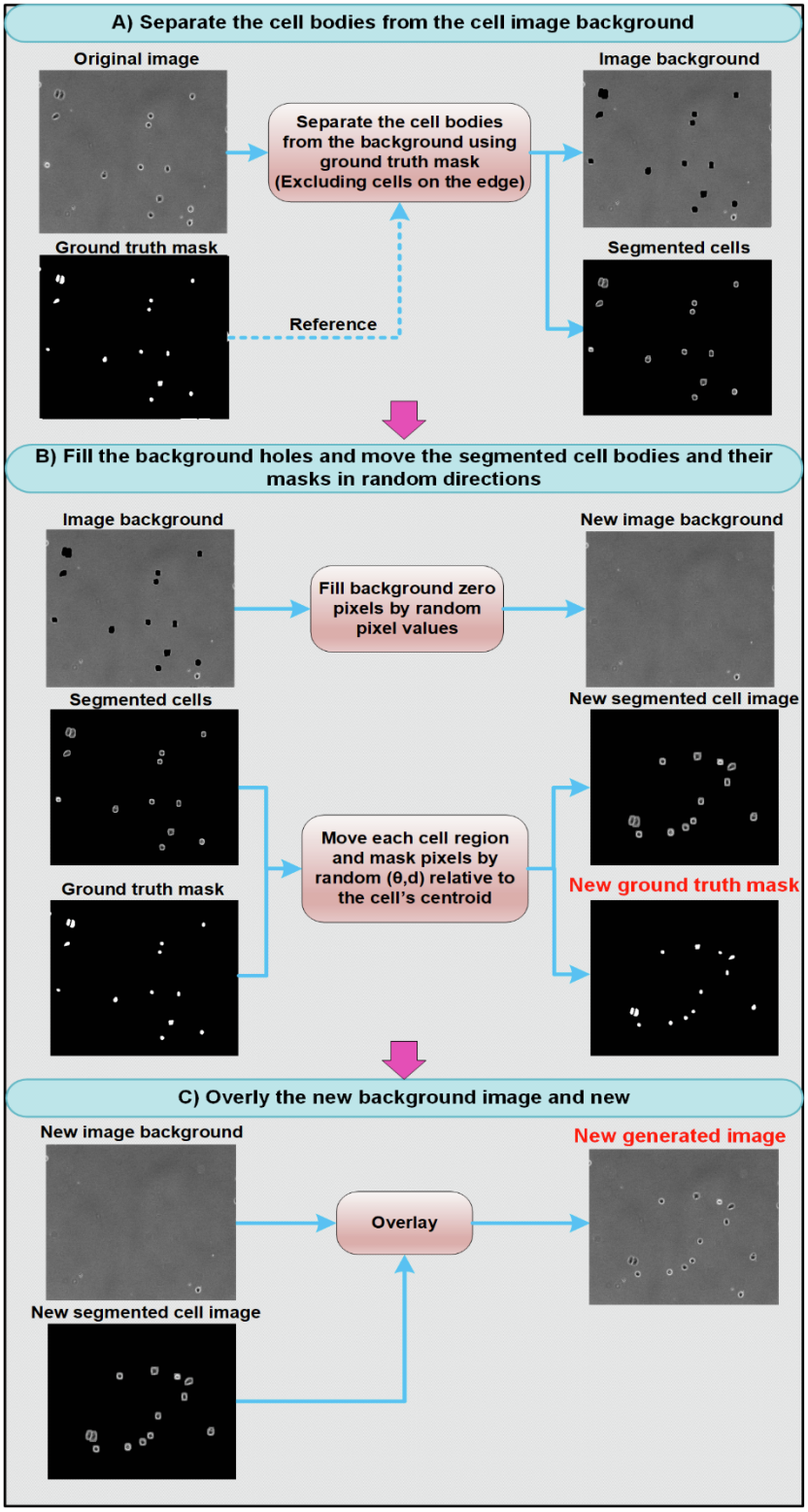

Figure S1. The proposed random cell movement block diagram, related to Figure 1 and Figure 2. It can more deeply perform cell image augmentation compared to conventional image augmentation methods. It generates new cell images with their annotated masks from the original

existing samples that look very different. θ is the direction angle between 0 and 360, and d is the displacement in pixels.

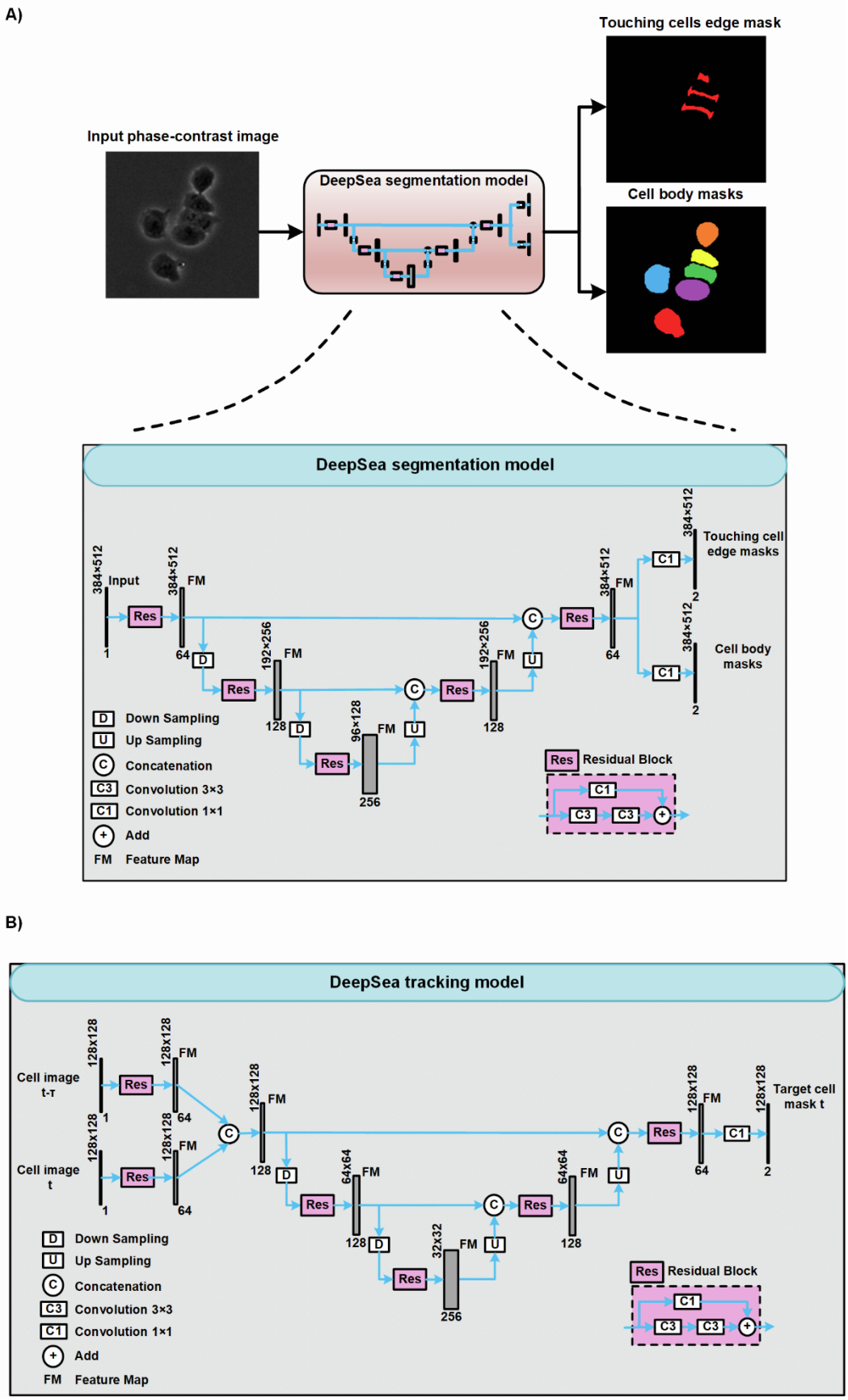
A)
Touching cells edge mask
Input phase-contrast image
DeepSea segmentation model
Cell body masks
DeepSea segmentation model
384×512
Input
1
384×512
FM
64
D
192×256
FM
128
D
96×128
FM
256
U
192×256
FM
128
U
C
Res
384×512
FM
64
C1
384×512
Touching cell edge masks
2
384×512
Cell body masks
2
D Down Sampling
U Up Sampling
C Concatenation
C3 Convolution 3×3
C1 Convolution 1×1
Add
FM Feature Map
Res Residual Block
B)
DeepSea tracking model
Cell image t-τ
Cell image t
128x128
FM
64
128
64x64
32x32
256
Target cell mask t
2
D Down Sampling
U Up Sampling
C Concatenation
C3 Convolution 3×3
C1 Convolution 1×1
Add
FM Feature Map
Res Residual Block

Figure S2. DeepSea segmentation and Tracking models, related to Figure 2, Figure 4, Figure 5. (A) The DeepSea segmentation model receives the label-free microscopy cell image and returns two outputs of the touching cell edge mask and the segmented cell body mask. This model architecture applies 1) a scaled-down version of 2D-UNET, 2) residual blocks to increase the depth of the model with fewer parameters, and 3) the auxiliary touching cell edge representations to improve the performance of the model, especially in high-density cell cultures. (B) DeepSea tracking model architecture with two input images of subsequent time points and the output of a binary mask localizing the target single cell on the current frame. Since it uses a segmentation-based process to localize and link the target cells across the frame sequences, we proposed an architecture similar to the DeepSea segmentation model as a fast and accurate enough architecture.

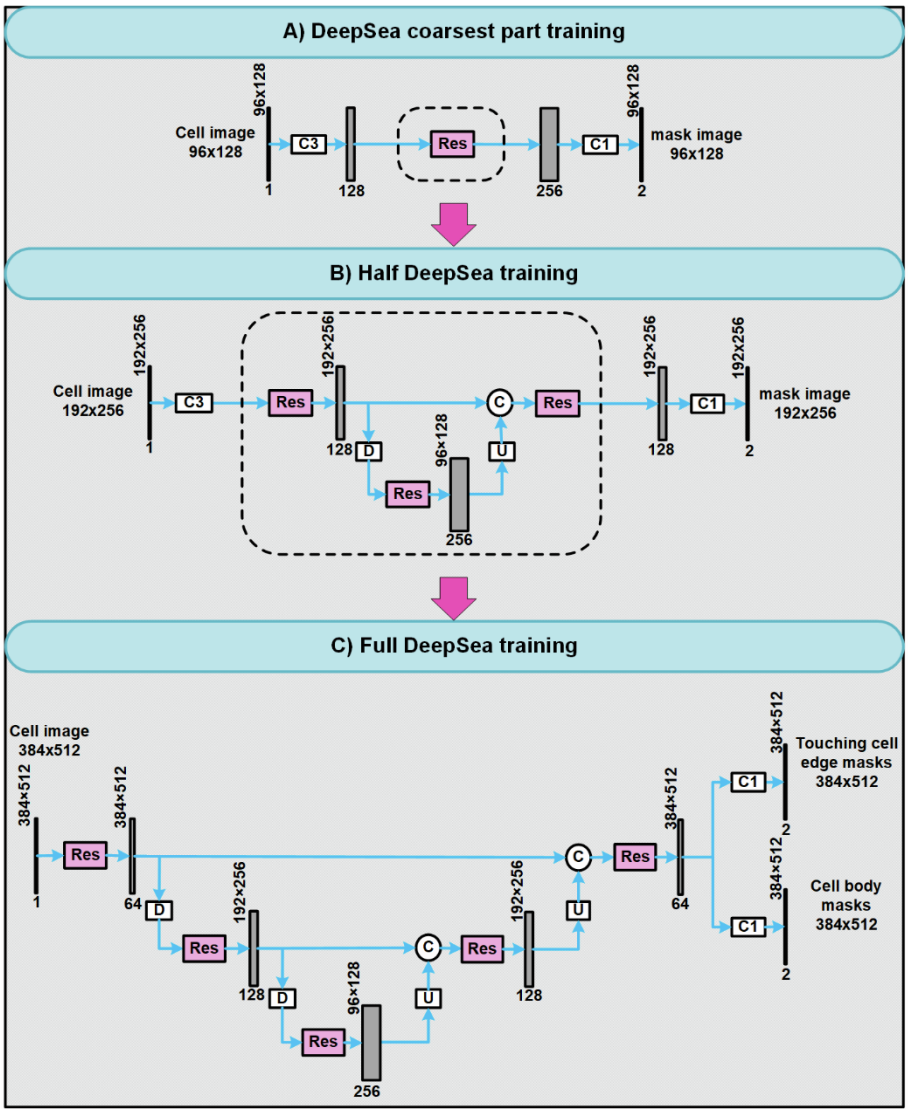

Figure S3. DeepSea progressive training stages, related to Figure 1, Figure 2, Figure 4, Figure 5. (A) First, the training algorithm starts training the coarsest part on low-resolution ground truth images of 96X128. (B) After some training epochs, it transfers the Res block weights to the half DeepSea model and keeps training it with the ground truth images of 192x256. (C) Finally, it finishes the last n training epochs with the full DeepSea model training.

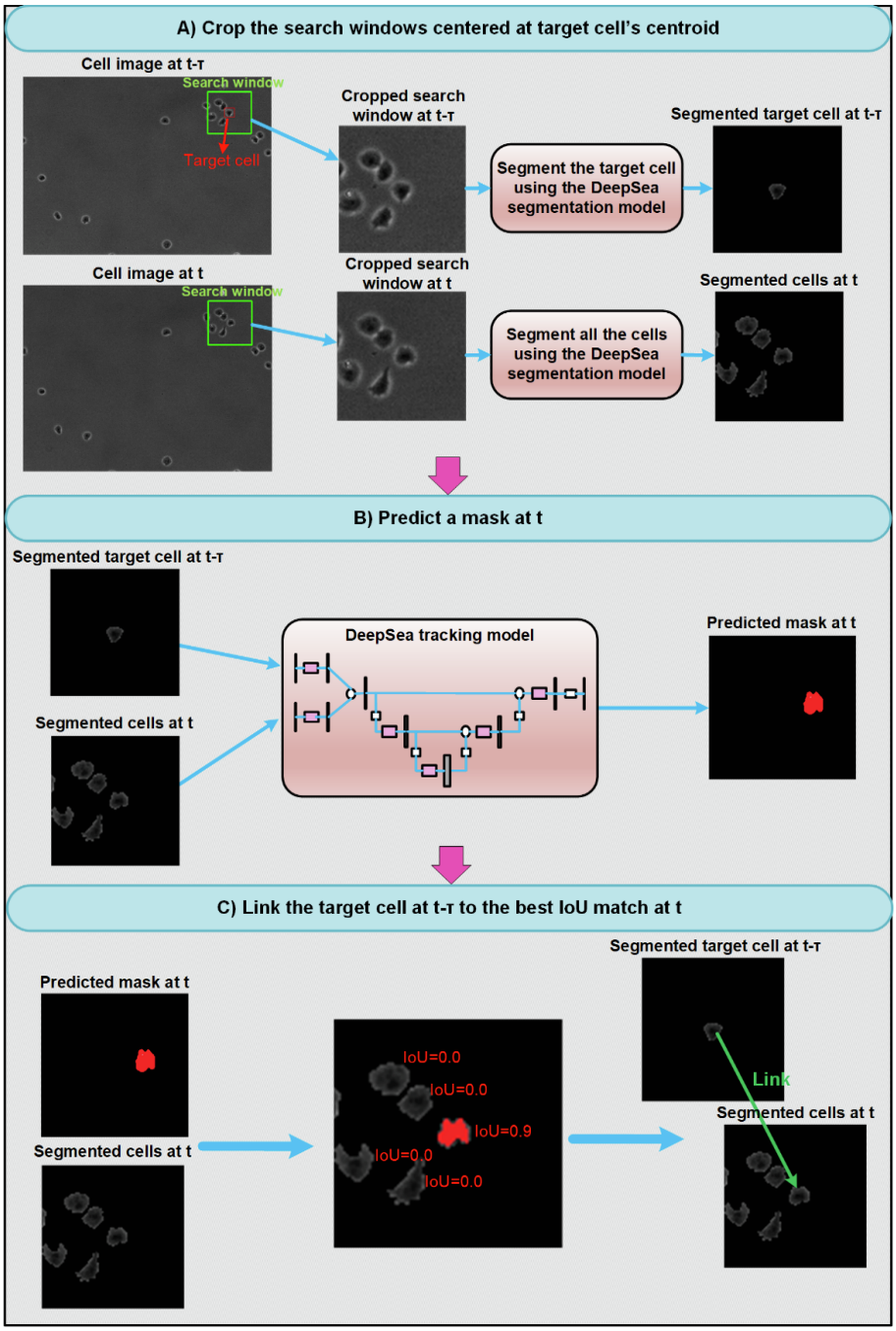

Figure S4. Single-cell tracking example from one frame to the next frame, related to Figure 4 and Figure 5. (A) We limited our search space in x and y coordinates to a small square with the size of 5 times the target cell size centered at the previous frame target cell's centroids. Then we fed each search crop into the DeepSea segmentation model to only have the segmented bodies of the target single cell on the previous frame and the segmented cells on the current frame. (B) The tracking model predicts the target single-cell location among the segmented cells on the current frame by generating a binary mask. (C) We validated the predictions using the IoU (Intersection over Union) score. We used the IoU score as a validation score to match the tracking model binary mask to each segmented cell body on the current frame and then find the true link

(target cell at t-τ to selected cell at t) corresponding to the highest IoU value. A valid IoU value should be higher than a pre-defined threshold value, e.g., IoU_thr=0.5. If the model finds two or more valid IoU values, it takes it as a mitosis occurrence and thus creates the mother-daughter links between the target cell of the previous frame and the two selected cells with the highest IoU values.

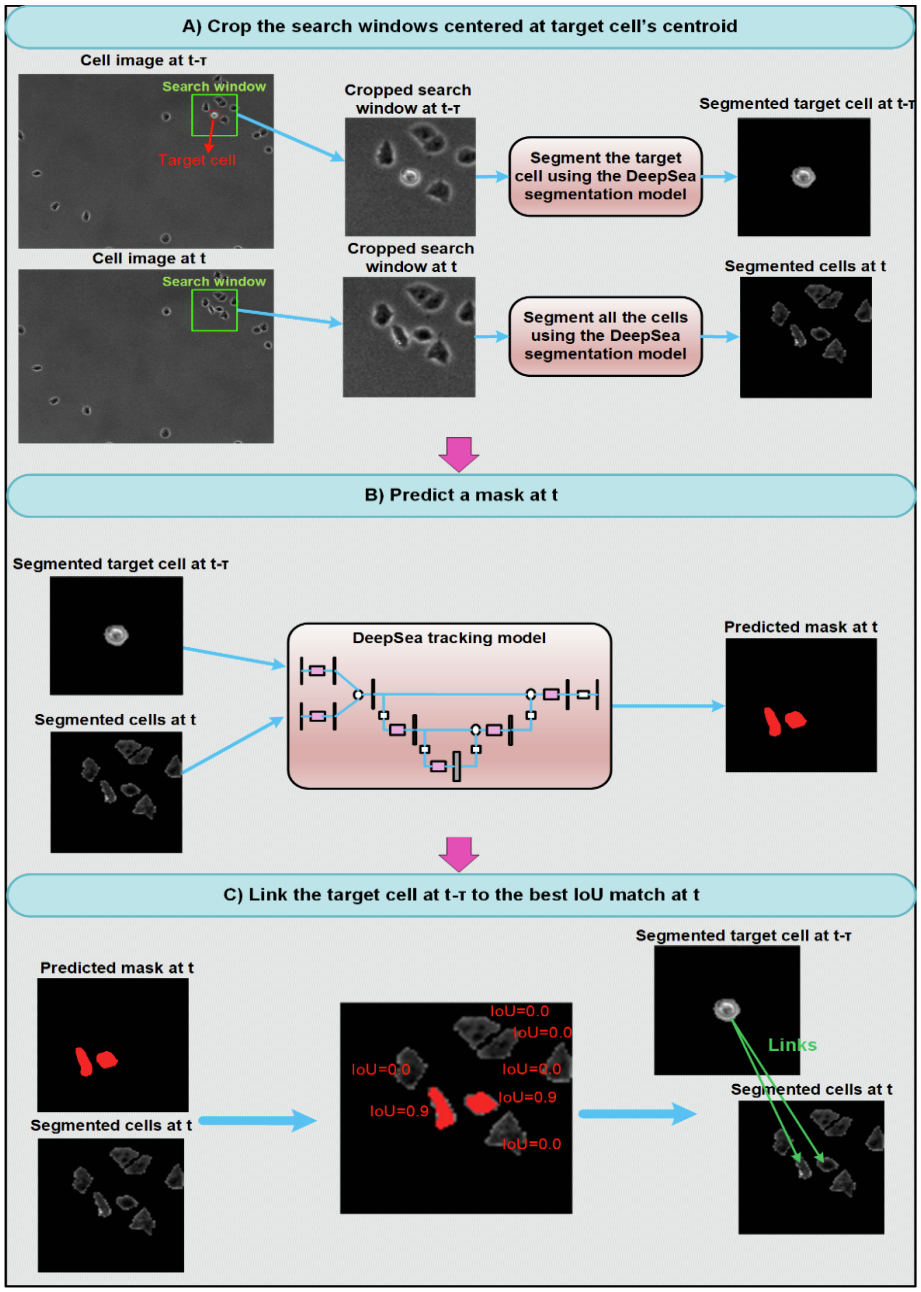

Figure S5. Daughter cell detection example from one frame to the next frame, related to Figure 4 and Figure 5 (A) We limited our search space in x and y coordinates to a small square with the size of 5 times the target cell size centered at the previous frame target cell's centroids. Then we fed each search crop into the DeepSea segmentation model to only have the segmented cell bodies of the target single cell on the previous frame and segmented cells on the current frame. (B) The tracking model predicts the daughter cell locations among the segmented cells on the current frame by generating a binary mask. (C) We validated the prediction using the IoU (Intersection over Union) score. We used the IoU as a validation score to match the tracking model binary mask to each segmented cell body on the current frame and then find the true mother-daughter links corresponding to the highest IoU values. A valid IoU value should be higher than a pre-defined threshold value, e.g., IoU_thr=0.5. If the model finds more than two valid IoU

values, it creates the mother-daughter links between the mother cell of the previous frame and the two selected cells with the highest IoU values. Also, if the model finds only one valid IoU value in the current frame, it takes it as a single-cell tracking (non-mitosis) event and thus creates a single link between the target cell of the previous frame and the single valid prediction on the current frame.

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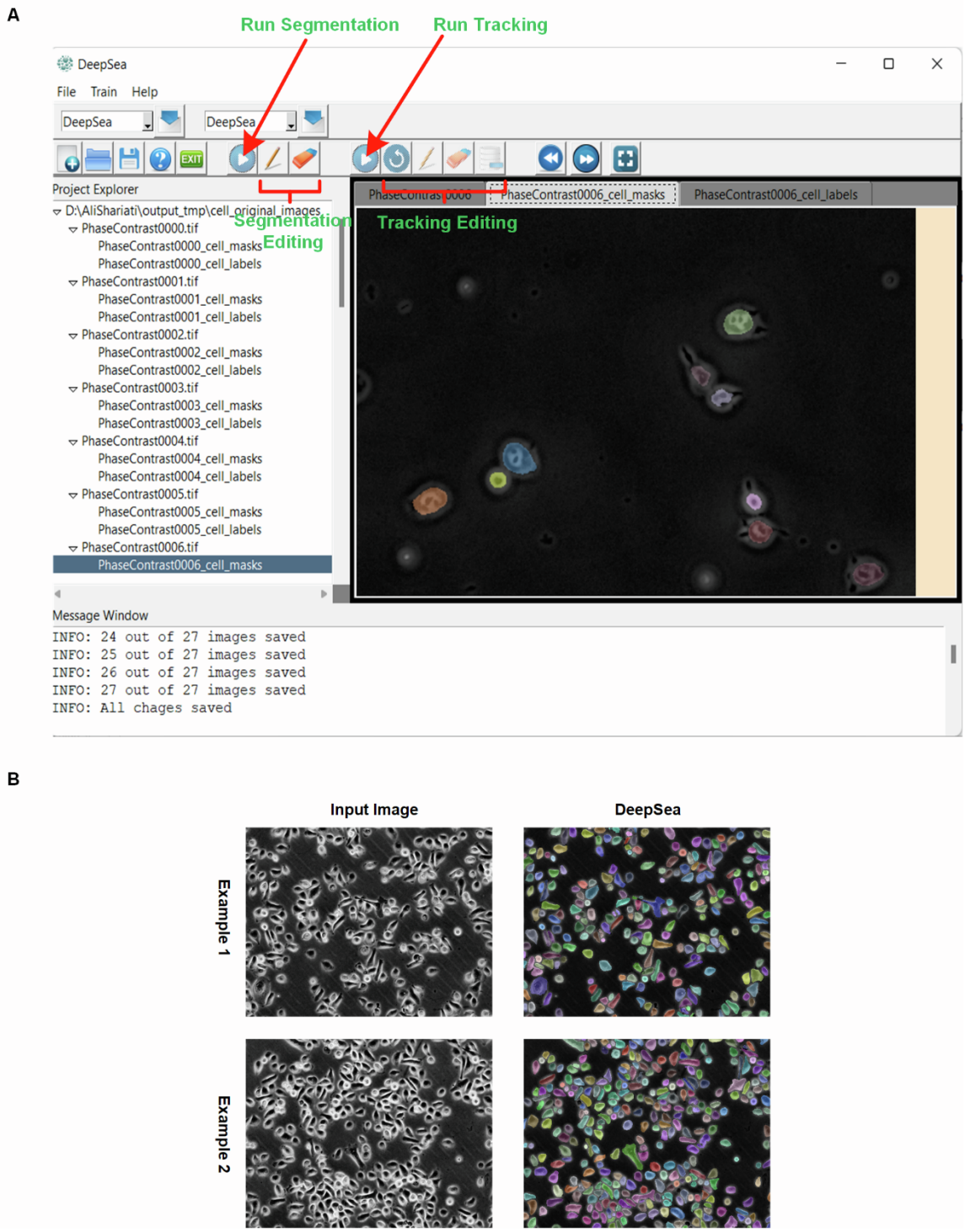

Figure S6. DeepSea software snapshot and examples of outputs, related to Figure 1 to Figure 5. (A) It is an automated cell segmentation and tracking software that employs our latest trained DeepSea models and also provides users the editing options to manually correct the DeepSea outputs. (B) Two examples of the DeepSea segmentation model output, confirming high precision with high-density cell images.

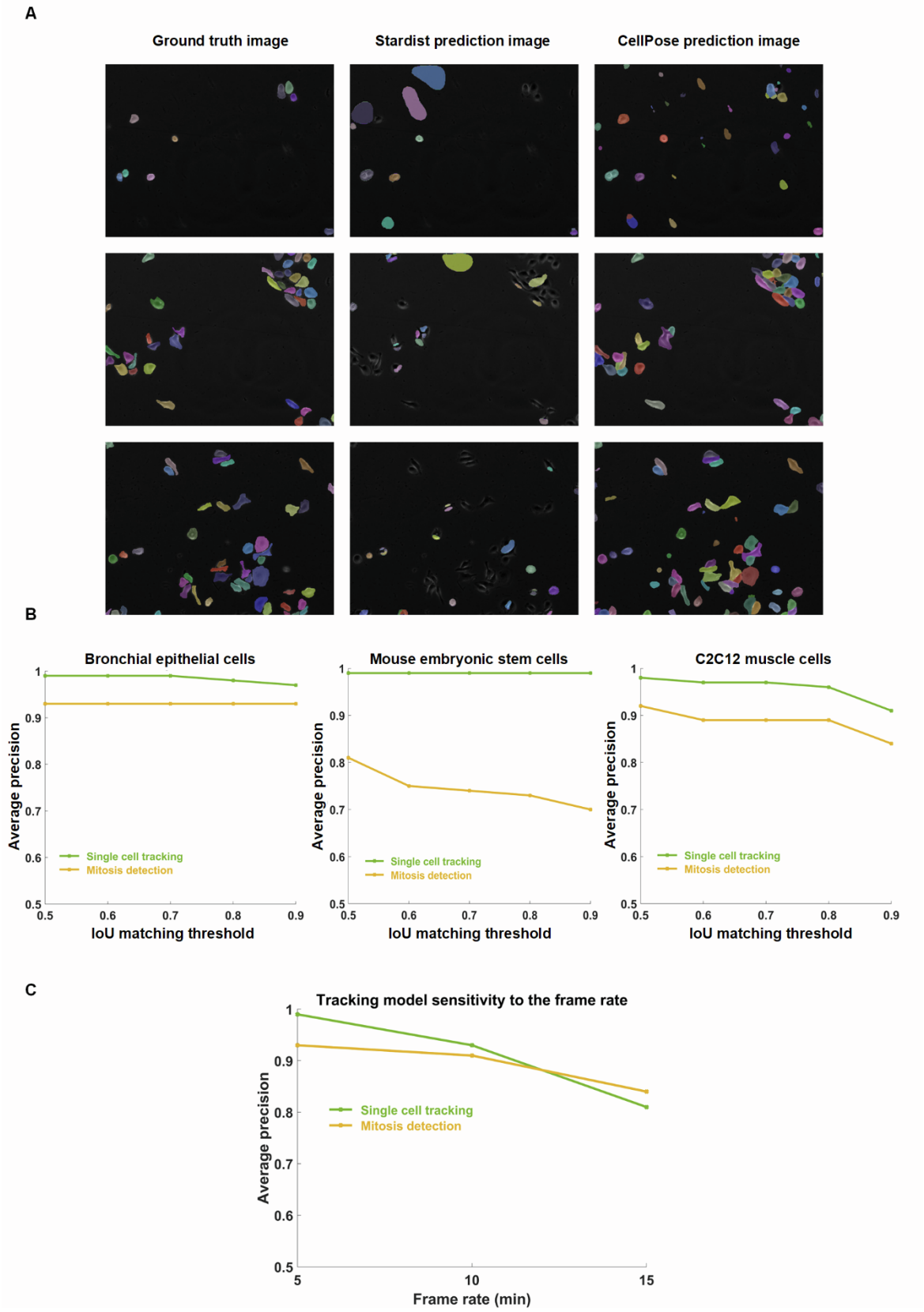
A
Ground truth image
Stardist prediction image
CellPose prediction image
B
Bronchial epithelial cells
Mouse embryonic stem cells
C2C12 muscle cells
Average precision
IoU matching threshold
Single cell tracking
Mitosis detection
C
Tracking model sensitivity to the frame rate
Frame rate (min)

Figure S7. Comparative performance analyses, related to Figure 1 to Figure 5. (A) The original pre-trained CellPose and StarDist model outputs compared with the ground truth cell body masks. (B) The DeepSea tracker model and mitotic detection performance with different cell types of the test set. (C) The tracking model sensitivity to the frame sampling rate. Bronchial epithelial cell images are used for this experiment.

Table S1. Model and training configurations, related to Figure 1 and Figrue 2.

	Easy samples ap@0.5	Hard samples ap@0.5	Number of parameters
2D UNET Scaled Down	0.87±0.1	0.82±0.2	1035778
Modified 2D UNET Scaled Down + Res Connections	0.93±0.1	0.84±0.1	1938306
Modified 2D UNET Scaled Down + Res Connections + Progressive Learning	0.94±0.2	0.87±0.2	1938306
Our DeepSea: Modified 2D UNET Scaled Down + Res Connections + Progressive Learning + EdgeDetectionLayer/Loss	0.93±0.2	0.90±0.1	1938436

Table S2. Single-cell tracking and mitosis detection precision, related to Figure 4 and Figure 5.

	Single-cell tracking	Mitosis detection
Trackmate[30]	0.76	0.36
CellTracker[27]	0.69	Not supported
MDMLM[29]	Not supported	0.85
CellTracking[28]	0.82	Not supported
DeepSea Tracker	0.98	0.89

Table S3. Multi-cell cycle tracking results, related to Figure 4 and Figure 5.

	MOTA	MT	ML	Precision	Recall	Frag	IDS	FP	FN	Number of cells	Number of trajectories	Latency Per image (GPU NVIDIA RTX2080)
Trackmate[30]	0.29	0.15	0.56	0.42	0.51	25	8	2614	2053	6624	228	85 ms
DeepSea Tracker	0.94	0.93	0.01	0.98	0.97	62	82	153	162	6624	228	580 ms

Table S4. DeepSea dataset characteristics, related to Figure 1 to Figure 5.

	Image Size	Frame Rate	Num of Sets	Num of Images	Num of Single Cells	Num of Cell Cycles
Stem Cells	1344x1024	15-30 min	30	2010	14995	115
Bronchial Cells	1244x904	5 min	8	1174	48027	292
Muscle Cells	1392x1040	20 min	9	502	22080	274