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Supplemental Information

Automated Deep Learning-Based System to Identify Endothelial Cells Derived from Induced Pluripotent Stem Cells

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Supplemental Figure Legends

Figure S1. Generation of iPSC-derived Endothelial Cells

(A) Differentiation of endothelial cells. iPSCs were seeded onto Matrigel-coated dishes, cultured in indicated conditions, and examined at day 6.

(B) Phase-contrast images at day 1 to 6. Scale bars, 500 μm.

(C) Phase-contrast images (upper panels), immunofluorescent staining for CD31 (middle panels), and FACS analysis (bottom panels) showed variability in differentiation at day 6 in various experiments. Left, middle, and right panels show experiments with high, intermediate, and low differentiation efficiency. Scale bars, 200 μm.

Figure S2. Network Performance Depending on Input Block Size, Staining Threshold and Target Block Size, Related to Figure 2, Tables S1 and S2.

(A) Phase-contrast and binarized fluorescent images of 512 × 512 px, 256 × 256 px, 128 × 128 px, 64 × 64 px, and 32 × 32 px blocks. Scale bars, 80 μm, 40 μm, 20 μm, 10 μm, and 5 μm, respectively.

(B) and (C) Accuracy obtained from networks trained on 32 × 32 px, 64 × 64 px, 128 × 128 px, 256 × 256 px, and 512 × 512 px input blocks, using 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 as staining threshold, *i.e.*, the ratio of white pixels to black for a binarized image to be classified as stained..

(D) F1 score and accuracy obtained from networks trained on input and target blocks of various sizes.

Figure S3. Optimization of Network Performance, Related to Figure 3.

(A) Learning curve of the small and large network, as assessed by accuracy.

(B) Representative images of true positives and true negatives (blue) and of false positives and false negatives (red). Yellow areas are CD31-stained. Scale bars, 200 μm.

Figure S4. Correlation Between Image Complexity and F1 score, Related to Figure 3.

(A) Representative phase-contrast images with complexity 0.00-0.04 (group 1), 0.04-0.08 (group 2), and over 0.08 (group 3). Scale bars, 80 μm.

(B) F1 score in each group using the small and large network (left), and relationship between F1 score and image complexity (right).

(C) and (D) Performance statistics from each group (C) and over increasing complexity (D). True positive: TP, True negative: TN, False positive: FP, and False negative: FN

(E) Time required to classify each block.

Supplemental Table Legends

Table S1. Number of Blocks Required for Learning, Related to Figure 2.

Networks were trained on 500, 1,000, 2,000, 4,000, 8,000, 16,000, 32,000, 64,000, and 128,000 blocks. Accuracy, recall, precision, and F1 score were assessed using 128 × 128 px input blocks and 128 × 128 px target blocks (left), or using 512 × 512 px input blocks and 32 × 32 px target blocks (right).

Table S2. Network Performance Depending on Input Block Size and Staining Threshold, Related to Figures 2 and S2.

Networks were trained using 32 × 32 px, 64 × 64 px, 128 × 128 px, 256 × 256 px, and 512 × 512 px input blocks, using 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 as staining threshold, *i.e.*, the ratio of white pixels to black for a binarized image to be classified as stained. Accuracy, recall, precision, and F1 score were calculated.

Table S3. Network Performance Depending on Target Block Size, Related to Figures 2 and S2.

F1 score, accuracy and other indices obtained from networks trained on input and target blocks of various sizes.

Table S4. Network Performance, Related to Figures 3 and 4.

(A) Network performance was compared following training on automatically binarized or rebinarized fluorescent images.

(B) K-fold cross validation of the small network trained on automatically binarized fluorescent images (left), and of the large network trained on rebinarized fluorescent images (right). Independent training and validation were performed according to Figure 4.

Supplemental Experimental Procedures

iPSC Culture

iPSCs were maintained in mTeSR1 (Stem Cell Technologies, Vancouver, BC, Canada) media with 0.5 % penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) on culture dishes coated with growth factor-reduced Matrigel (BD Biosciences, San Jose, CA, USA). iPSCs were routinely passaged every week by washing in PBS, incubating in TrypLE Select (Thermo Fisher Scientific) for 3 min at 37 °C, detaching with a cell scraper, harvesting, and reseeding at a split ratio of 1:5 to 1:8 in mTeSR1 with 0.5 % penicillin/streptomycin and 10 μM ROCK inhibitor Y-27632 (Wako, Osaka, Japan). Media were changed every other day.

Endothelial Cell Differentiation

iPSCs cultured on Matrigel-coated 6-well plates were detached using TrypLE Select on day 7, and clumps with diameter 100-200 μm were reseeded on Matrigel-coated dishes and incubated for 24 hours in mTeSR1 media with 10 μM ROCK inhibitor Y-27632. On day 1, mesoderm was induced in N2B27 media (1:1 mixture of DMEM/F12 and Neurobasal media containing N2 and B27, all reagents from Thermo Fisher Scientific) supplemented with β-mercaptoethanol, 8 μM CHIR-99021 (Cayman Chemical, Ann Arbor, MI, USA), and 25 ng/mL BMP4 (R&D Systems, Minneapolis, MN, USA). At day 3 and 4, media were replaced with StemPro-34 SFM (Thermo Fisher Scientific) containing 200 ng/mL VEGF (Wako) and 2 μM forskolin (Abcam, Cambridge, UK) to induce endothelial cell specification (Patsch et al., 2015). Endothelial cell clusters were reliably obtained on day 6. After sorting by flow cytometry, cells expressing CD31 were cultured for another four days in StemPro-34 SFM containing 50 ng/mL VEGF.

Flow Cytometry

At day 6 of differentiation, cells were dissociated into singe cells using Accutase (Innovative Cell Technologies, San Diego, CA, USA), suspended in PBS with 0.5 % BSA, and stained with a 1:50 dilution of APC-conjugated anti-CD31 (Miltenyi Biotec, Bergisch Gladbach, NRW, Germany, catalog no. 130-092-652) according to the manufacturer's instructions. As a negative control, we used unstained cells. Cells were then sorted on a BD FACS Aria III (Becton Dickinson, Franklin Lakes, NJ, USA), and data were collected from at least 10,000 events.

Immunocytochemistry

Cells were fixed in 4 % paraformaldehyde (MUTO Pure Chemicals, Tokyo, Japan) for 20 min at room temperature, washed with PBS, blocked with ImmunoBlock (DS Pharma Biomedical, Osaka, Japan) for 1 h, and probed at 4 °C overnight with 1:20 primary antibodies to CD31 (R&D Systems, catalog no. AF806). Specimens were then washed thrice in PBS, labeled for 1 h with 1:200 secondary anti-sheep IgG (Thermo Fisher Scientific, catalog no. A-11015), and imaged on an inverted fluorescence phase-contrast microscope.

Preparation of Datasets

Phase-contrast and immunofluorescent images were acquired at day 6 of differentiation. Two hundred images were automatically acquired from each of four independent experiments. Phase contrast and fluorescent images were taken on an SI8000 Research Microscope (SONY, Tokyo, Japan) at 10× and 0.454 μm/pixel. Each image was saved as a

2752 × 2200 px grayscale image in BMP format at 8 bits per pixel. To generate datasets for training and evaluation, 200 input blocks of 32 × 32 px, 64 × 64 px, 128 × 128 px, 256 × 256 px, and 512 × 512 px were randomly extracted from each phase-contrast image. The 256 × 256 px and 512 × 512 px input blocks were resized to 128 × 128 px as needed. Immunofluorescent images of CD31 were binarized using in-house software to distinguish specific signals from nonspecific signals. In particular, pixels were binarized to white if its value (0-255 in raw immunofluorescent images) is above a threshold value empirically determined based on the complete image. All other pixels were binarized to black. Finally, 32 × 32 px and 128 × 128 px target blocks were extracted, corresponding to the center of input blocks.

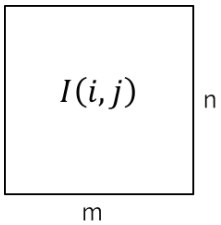
Data in Figure 2 and 3 were generated based on 640 training images and 160 validation images. In both experiments in Figure 2A, 500, 1,000, 2,000, 4,000, 8,000, 16,000, 32,000, 64,000, and 128,000 blocks were used for training, and 32,000 blocks were used for validation. In Figure 2B, 32,000 blocks were used for training, and 32,000 blocks were used for validation. In Figure 2C to 3E, all 128,000 blocks were used for training, and 32,000 blocks were used for validation. For K-fold validation in Figure 4, four independent data sets of 200 images each were obtained, of which three were used as training sets and one was used as validation set in all possible combinations, such that the number of folds is 4. To rebinarize target blocks, we compared raw fluorescent images to phase-contrast images in GNU Image Manipulation Program, and rebinarized weakly stained, dense colonies as black pixels. All 800 images were processed in this manner.

Deep Neural Networks

We used LeNet, a small convolutional neural network with two convolution layers, two max pooling layers, and two fully-connected layers, as well as AlexNet, a large network with five convolution layers, three max pooling layers, and three fully-connected layers (Figure 3A). In both networks, each convolutional layer is connected to Rectified Linear Units for activation (Nair and Hinton, 2010). In the output layer, we used a sigmoid function, consistent with binary classification. We used mini-batch training with stochastic gradient descent, learning rate 0.01, cross-entropy error as loss function. Weights were initialized using the Xavier algorithm (Glorot and Bengio, 2010). To avoid overfitting, dropout techniques were used in the large network. Networks were trained using the TensorFlow/Keras framework (Cholle, 2015) on a computer with a Core i7-6700 CPU (Intel, Santa Clara, CA, USA), 16 GB memory, and GeForce GTX980Ti GPU (NVIDIA, Santa Clara, CA, USA).

Image Complexity

We calculated image complexity (activity), which we used as an index of cell density, in all 32,000 512 × 512 px validation blocks used in the small and large network. This value was



$$Activity = \frac{\sum_{i=0}^{m-2}\sum_{j=0}^{n-1}|I(i,j) - I(i+1,j)| + \sum_{i=0}^{m-1}\sum_{j=0}^{n-2}|I(i,j) - I(i,j+1)|}{\{(\mathrm{m}-1)\mathrm{n} + \mathrm{m}(\mathrm{n}-1)\}\{\max(I) - \min(I)\}}$$

calculated according to

where m is the image width in pixels, n is the image height in pixels, *I* is the pixel value, and *(i, j)* are coordinates (x-axis, y-axis). Essentially, image complexity is the average difference between adjacent pixels normalized to the dynamic range (Saha and Vemuri, 2000). Thus, the numerator is the sum of differences in adjacent pixels on both x

and y axes, while the denominator is the product of image size and dynamic range, which is the difference between the maximum and minimum pixel value.

Evaluation of Prediction Performance

Network performance was evaluated based on accuracy and F1 score, which combines recall (sensitivity) and precision (true positive rate). Accordingly, the F1 score is 1 for perfect predictions and 0.5 for random predictions. On the other hand, precision is the fraction of true positives among predicted positives, while recall is the fraction of true positives detected among all positives:

$$F1\ score\ = \frac{2Recall\ \times\ Precision}{Recall + Precision}, Precision\ = \frac{TP}{TP + FP}, Recall\ = \frac{TP}{TP + FN}$$

Precision and recall for negative predictions were calculated in a similar manner:

$$Precision\ (negative) = \frac{TN}{TN + FN}, Recall\ (negative)\ = \frac{TN}{TN + FP}$$

Finally, accuracy is the ratio of correct predictions to all predictions:

$$Accuracy = \frac{TP + TN}{TP + FP + TN +\ FP}$$

Supplemental References

Cholle, F. (2015). Keras (https://github.com/fchollet/keras: GitHub).

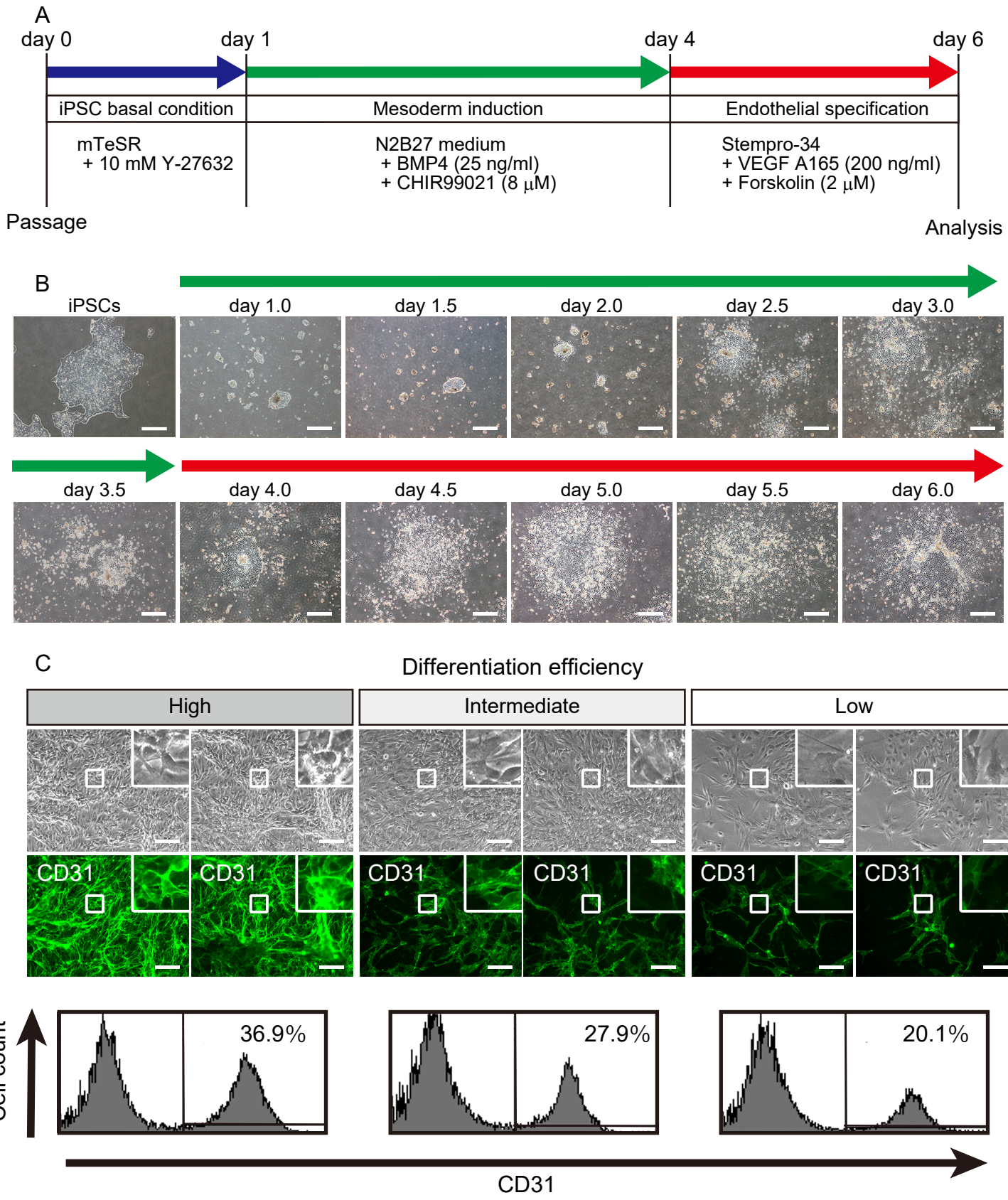
Glorot, X., and Bengio, Y. (2010). Understanding the difficulty of training deep feedforward neural networks. In Proceedings of the Thirteenth International Conference on Artificial Intelligence and Statistics, T. Yee Whye, and T. Mike, eds. (Proceedings of Machine Learning Research: PMLR), pp. 249--256.

Nair, V., and Hinton, G.E. (2010). Rectified linear units improve restricted Boltzmann machines. In Proceedings of the 27th International Conference on International Conference on Machine Learning (Haifa, Israel: Omnipress), pp. 807-814.

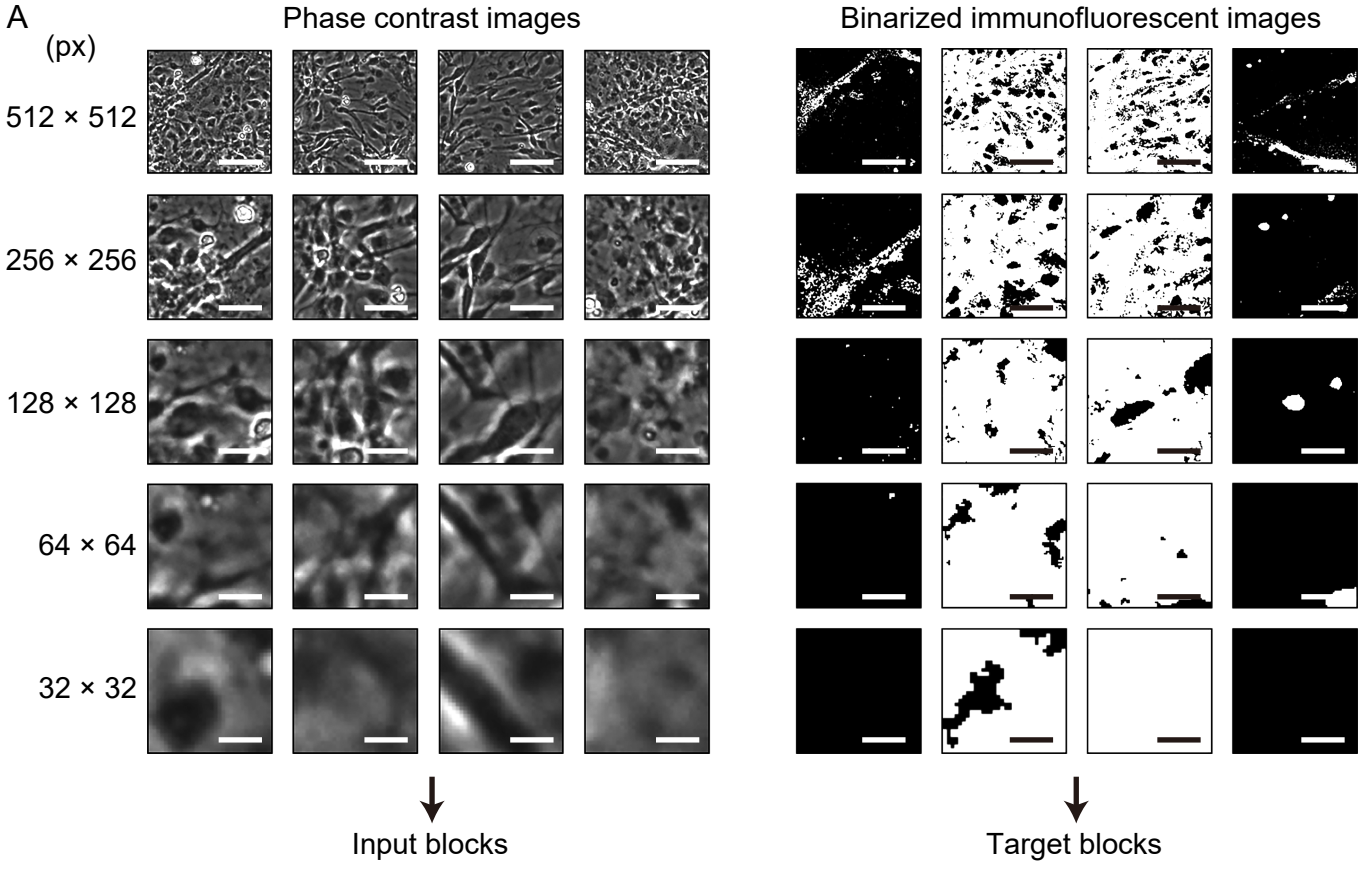
Patsch, C., Challet-Meylan, L., Thoma, E.C., Urich, E., Heckel, T., O'Sullivan, J.F., Grainger, S.J., Kapp, F.G., Sun, L., Christensen, K.*, et al.* (2015). Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells. Nat Cell Biol *17*, 994-1003.

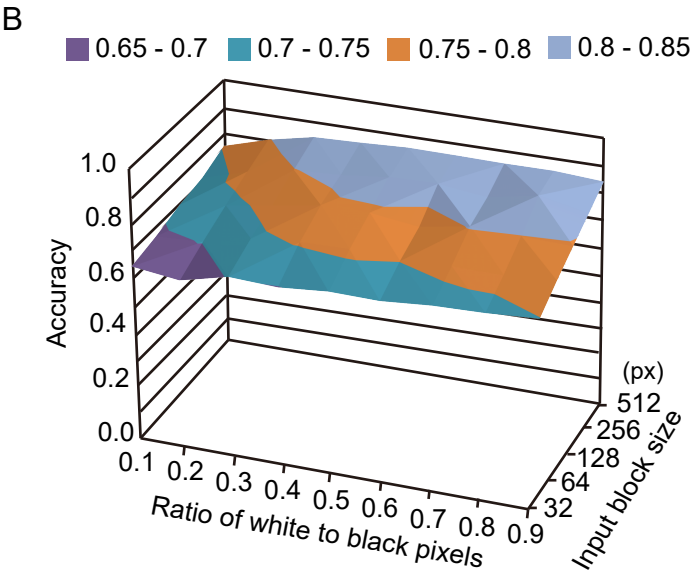
Saha, S., and Vemuri, R. (2000). An analysis on the effect of image activity on lossy coding performance. Paper presented at: 2000 IEEE International Symposium on Circuits and Systems Emerging Technologies for the 21st Century Proceedings (IEEE Cat No00CH36353).

Supplementary Figure 1



Supplementary Figure 2



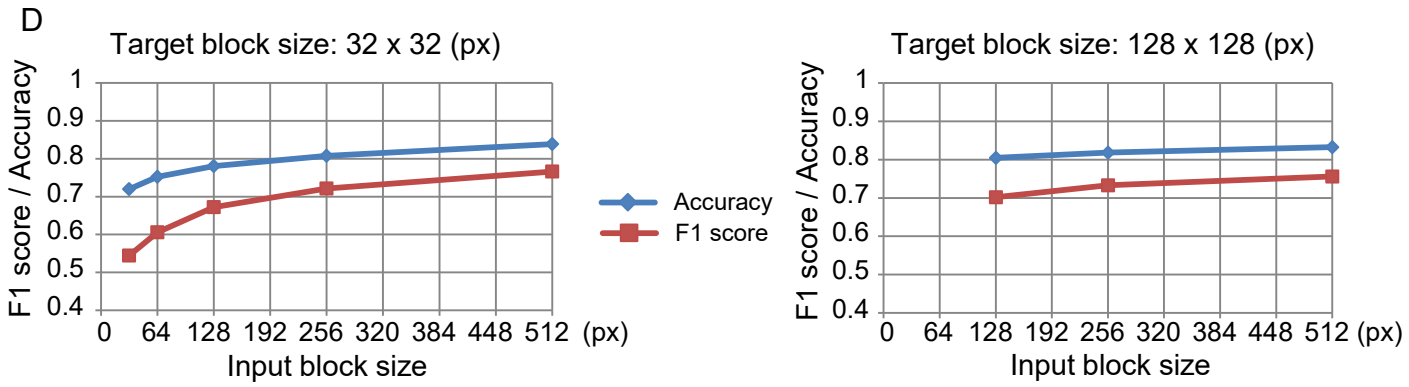


C

Accuracy

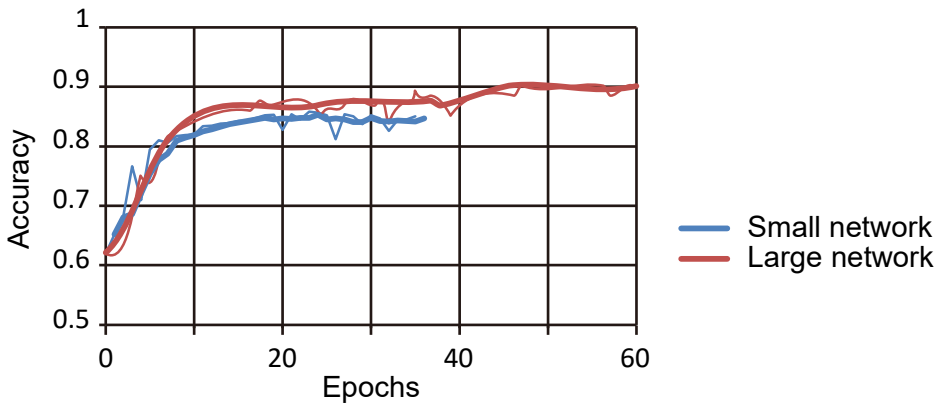
Input block size (px)

Ratio of white to black pixels	32	64	128	256	512	Average
0.1	0.6786	0.6919	0.7121	0.7230	0.7529	0.7117
0.2	0.6624	0.7019	0.7364	0.7685	0.7978	0.7334
0.3	0.7028	0.7389	0.7626	0.7988	0.8304	0.7667
0.4	0.6986	0.7546	0.7900	0.8084	0.8366	0.7776
0.5	0.7144	0.7599	0.7925	0.8258	0.8448	0.7875
0.6	0.7143	0.7509	0.7745	0.8304	0.8444	0.7829
0.7	0.7295	0.7635	0.8027	0.8140	0.8431	0.7906
0.8	0.7364	0.7639	0.7994	0.8448	0.8448	0.7979
0.9	0.7491	0.7752	0.7954	0.8147	0.8360	0.7941
Average	0.7096	0.7445	0.7740	0.8032	0.8256	

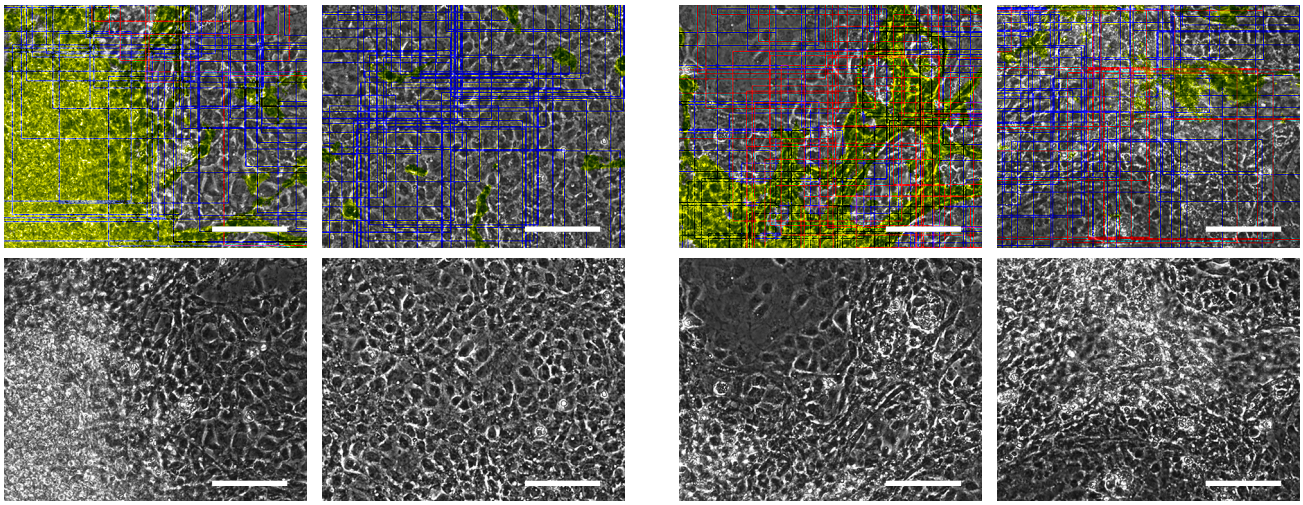


Supplementary Figure 3

A

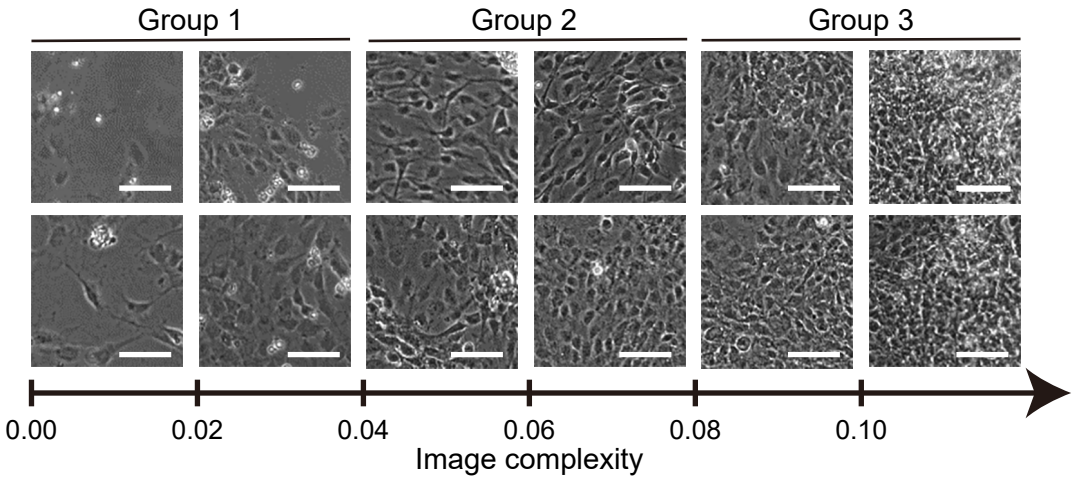


B



Supplementary Figure 4

A



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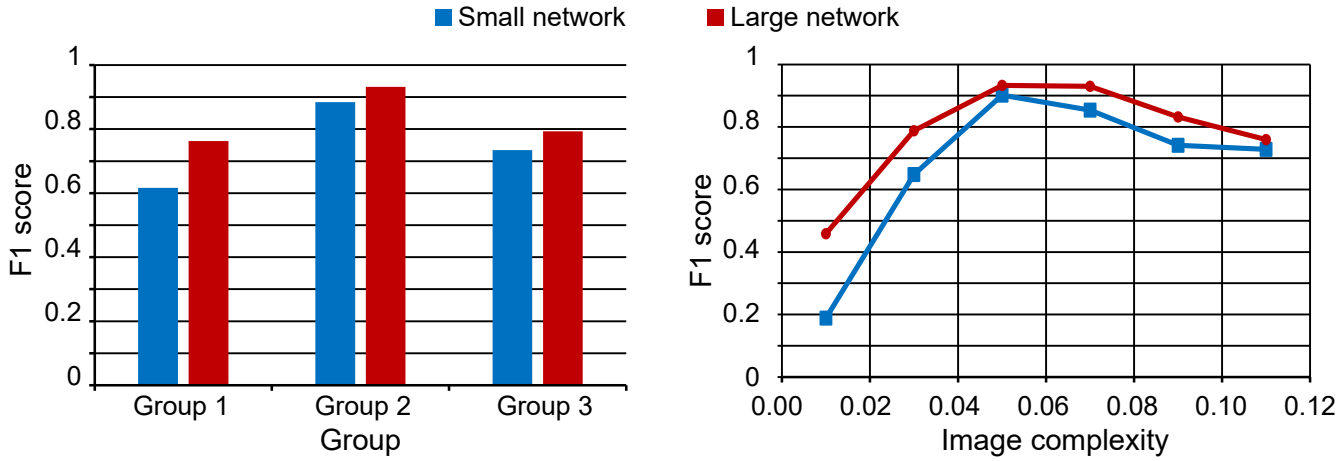

C

Small network

Group	Image complexity	TN	FN	FP	TP	Precision	Recall	Accuracy	F1 score
1	0.00-0.04	8,657	707	546	1,006	0.648196	0.587274	0.885214	0.616233
2	0.04-0.08	2,758	992	633	6,192	0.907253	0.861915	0.846336	0.884003
3	0.08-	6,628	1,025	604	2,252	0.788515	0.687214	0.84499	0.734388

Large network

Group	Image complexity	TN	FN	FP	TP	Precision	Recall	Accuracy	F1 score
1	0.00-0.04	8,870	452	333	1,261	0.791092	0.736135	0.928087	0.762625
2	0.04-0.08	2,818	413	573	6,771	0.921977	0.942511	0.906761	0.932131
3	0.08-	6,603	709	629	2,568	0.803253	0.783644	0.872681	0.793327

D

Small network

Image complexity	TN	FN	FP	TP	Precision	Recall	Accuracy	F1 score
0.00-0.02	4,595	165	16	21	0.567568	0.112903	0.962268	0.188341
0.02-0.04	4,062	542	530	985	0.650165	0.645056	0.824808	0.6476
0.04-0.06	1,105	475	395	3,991	0.909941	0.893641	0.854174	0.901717
0.06-0.08	1,653	517	238	2,201	0.902419	0.809787	0.83619	0.853597
0.08-0.10	3,078	487	219	1,014	0.822384	0.67555	0.852855	0.74177
0.10-	3,550	538	385	1,238	0.762785	0.697072	0.838382	0.72845

Large network

Image complexity	TN	FN	FP	TP	Precision	Recall	Accuracy	F1 score
0.00-0.02	4,602	128	9	58	0.865672	0.311828	0.97144	0.458498
0.02-0.04	4,268	324	324	1,203	0.787819	0.787819	0.8941	0.787819
0.04-0.06	1,149	251	351	4,215	0.923127	0.943798	0.899095	0.933348
0.06-0.08	1,669	162	222	2,556	0.920086	0.940397	0.916685	0.930131
0.08-0.10	3,039	247	258	1,254	0.829365	0.835443	0.894748	0.832393
0.10-	3,564	462	371	1,314	0.779822	0.739865	0.854141	0.759318

E

Small network: 100 μsec
Large network: 275 μsec

Supplementary Table 1

Pred = “Prediction” ; Ans = “Answer” ; 0 = “unstained” ; 1 = “stained”

Number of input blocks

	Input block size: 128 x 128 (px) Target block size: 128 x 128 (px)				Input block size: 512 x 512 (px) Target block size: 32 x 32 (px)			
500		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	21,261	0	21,261	1	21,148	0	21,148	1
	Ans=1	10,739	0	10,739	0	10,852	0	10,852	0
	Total	32,000	0	32,000		32,000	0	32,000	
	Precision	0.6644	0			0.6609	0		
	F1 score	0.7984	0			0.7958	0		
	Accuracy	0.6644				0.6609			
1,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	21,261	0	21,261	1	21,148	0	21,148	1
	Ans=1	10,737	2	10,739	0.0002	10,852	0	10,852	0
	Total	31,998	2	32,000		32,000	0	32,000	
	Precision	0.6644	1			0.6609	0		
	F1 score	0.7984	0.0004			0.7958	0		
	Accuracy	0.6645				0.6609			
2,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	21,261	0	21,261	1	21,129	19	21,148	0.9991
	Ans=1	10,738	1	10,739	0.0001	10,774	78	10,852	0.0072
	Total	31,999	1	32,000		31,903	97	32,000	
	Precision	0.6644	1			0.6623	0.8041		
	F1 score	0.7984	0.0002			0.7966	0.0142		
	Accuracy	0.6644				0.6627			
4,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	20,913	348	21,261	0.9836	18,684	2,464	21,148	0.8835
	Ans=1	9,901	838	10,739	0.078	8,276	2,576	10,852	0.2374
	Total	30,814	1,186	32,000		26,960	5,040	32,000	
	Precision	0.6787	0.7066			0.693	0.5111		
	F1 score	0.8032	0.1405			0.7768	0.3242		
	Accuracy	0.6797				0.6644			
8,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	18,933	2,328	21,261	0.8905	18,836	2,312	21,148	0.8907
	Ans=1	6,646	4,093	10,739	0.3811	6,514	4,338	10,852	0.3997
	Total	25,579	6,421	32,000		25,350	6,650	32,000	
	Precision	0.7402	0.6374			0.743	0.6523		
	F1 score	0.8084	0.477			0.8102	0.4957		
	Accuracy	0.7196				0.7242			
16,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	19,298	1,963	21,261	0.9077	18,816	2,332	21,148	0.8897
	Ans=1	5,765	4,974	10,739	0.4632	4,113	6,739	10,852	0.621
	Total	25,063	6,937	32,000		22,929	9,071	32,000	
	Precision	0.77	0.717			0.8206	0.7429		
	F1 score	0.8332	0.5628			0.8538	0.6765		
	Accuracy	0.7585				0.7986			
32,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	18,649	2,612	21,261	0.8771	18,743	2,405	21,148	0.8863
	Ans=1	4,635	6,104	10,739	0.5684	3,865	6,987	10,852	0.6438
	Total	23,284	8,716	32,000		22,608	9,392	32,000	
	Precision	0.8009	0.7003			0.829	0.7439		
	F1 score	0.8373	0.6275			0.8567	0.6903		
	Accuracy	0.7735				0.8041			
64,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	19,007	2,254	21,261	0.894	18,754	2,394	21,148	0.8868
	Ans=1	4,019	6,720	10,739	0.6258	3,225	7,627	10,852	0.7028
	Total	23,026	8,974	32,,000		21,979	10,021	32,000	
	Precision	0.8255	0.7488			0.8533	0.7611		
	F1 score	0.8584	0.6818			0.8697	0.7308		
	Accuracy	0.804				0.8244			
128,000		Pred=0	Pred=1	Total	Recall	Pred=0	Pred=1	Total	Recall
	Ans=0	18,409	2,852	21,261	0.8659	18,558	2,590	21,148	0.8775
	Ans=1	3,388	7,351	10,739	0.6845	2,730	8,122	10,852	0.7484
	Total	21,797	10,203	32,000		21,288	10,712	32,000	
	Precision	0.8446	0.7205			0.8718	0.7582		
	F1 score	0.8551	0.702			0.8746	0.7533		
	Accuracy	0.805				0.8337			

Supplementary Table 2

Pred = “Prediction” : 0 = “unstained” , 1 = “stained”
Ans = “Answer” : 0 = “unstained” , 1 = “stained”

Input block size (px)

Ratio of white to black pixels

0.1

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	16,467	2,885	19,352	0.8509
Ans=1	7,401	5,247	12,648	0.4148
Total	23,868	8,132	32,000	
Precision	0.6899	0.6452		
F1 score	0.762	0.505		
Accuracy	0.6786			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	14,370	4,256	18,626	0.7715
Ans=1	5,602	7,772	13,374	0.5811
Total	19,972	120,28	32,000	
Precision	0.7195	0.6462		
F1 score	0.7446	0.6119		
Accuracy	0.6919			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	13,616	4,235	17,851	0.7628
Ans=1	4,979	9,170	14,149	0.6481
Total	18,595	13,405	32,000	
Precision	0.7322	0.6841		
F1 score	0.7472	0.6656		
Accuracy	0.7121			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	14,587	2,392	16,979	0.8591
Ans=1	6,471	8,550	15,021	0.5692
Total	21,058	10,942	32,000	
Precision	0.6927	0.7814		
F1 score	0.767	0.6586		
Accuracy	0.723			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	13,447	2,771	16,218	0.8291
Ans=1	5,137	10,645	15,782	0.6745
Total	18,584	13,416	32,000	
Precision	0.7236	0.7935		
F1 score	0.7728	0.7292		
Accuracy	0.7529			

0.2

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	13,946	5,999	19,945	0.6992
Ans=1	4,805	7,250	12,055	0.6014
Total	18,751	13,249	32,000	
Precision	0.7437	0.5472		
F1 score	0.7208	0.573		
Accuracy	0.6624			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	14,719	4,817	19,536	0.7534
Ans=1	4,721	7,743	12,464	0.6212
Total	19,440	12,560	32,000	
Precision	0.7572	0.6165		
F1 score	0.7553	0.6188		
Accuracy	0.7019			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	15,570	3,594	19,164	0.8125
Ans=1	4,840	7,996	12,836	0.6229
Total	20,410	11,590	32,000	
Precision	0.7629	0.6899		
F1 score	0.7869	0.6547		
Accuracy	0.7364			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	16,199	2,723	18,922	0.8561
Ans=1	4,685	8,393	13,078	0.6418
Total	20,884	11,116	32,000	
Precision	0.7757	0.755		
F1 score	0.8139	0.6938		
Accuracy	0.7685			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	15,390	3,067	18,457	0.8338
Ans=1	3,403	10,140	13,543	0.7487
Total	18,793	13,207	32,000	
Precision	0.8189	0.7678		
F1 score	0.8263	0.7581		
Accuracy	0.7978			

0.3

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	16,304	4,081	20,385	0.7998
Ans=1	5,430	6,185	11,615	0.5325
Total	21,734	10,266	32,000	
Precision	0.7502	0.6025		
F1 score	0.7742	0.5653		
Accuracy	0.7028			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	17,128	3,042	20,170	0.8492
Ans=1	5,314	6,516	11,830	0.5508
Total	22,789	9,211	32,000	
Precision	0.7632	0.6817		
F1 score	0.8039	0.6093		
Accuracy	0.7389			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	17,085	2,975	20,060	0.8517
Ans=1	4,623	7,317	11,940	0.6128
Total	21,708	102,92	32,000	
Precision	0.787	0.7109		
F1 score	0.8181	0.6582		
Accuracy	0.7626			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	17,001	3,035	20,036	0.8485
Ans=1	3,404	8,560	11,964	0.7155
Total	20,405	11,595	32,000	
Precision	0.8332	0.7382		
F1 score	0.8408	0.7267		
Accuracy	0.7988			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	17,963	1,863	19,826	0.906
Ans=1	3,564	8,610	12,174	0.7072
Total	21,527	10,473	32,000	
Precision	0.8344	0.8221		
F1 score	0.8688	0.7604		
Accuracy	0.8304			

0.4

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	17,514	3,227	20,741	0.8444
Ans=1	6,419	4,840	11,259	0.4299
Total	23,933	8,067	32,000	
Precision	0.7318	0.6		
F1 score	0.7841	0.5009		
Accuracy	0.6986			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	17,807	2,872	20,679	0.8611
Ans=1	4982	6,339	11,321	0.5599
Total	22,789	9,211	32,000	
Precision	0.7814	0.6882		
F1 score	0.8193	0.6175		
Accuracy	0.7546			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	17,912	2,776	20,688	0.8658
Ans=1	3,943	7,369	11,312	0.6514
Total	21,855	10,145	32000	
Precision	0.8196	0.7264		
F1 score	0.8421	0.6869		
Accuracy	0.79			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	18,601	2,219	20,820	0.8934
Ans=1	3,913	7,267	11,180	0.65
Total	22,514	9,486	32,000	
Precision	0.8262	0.7661		
F1 score	0.8585	0.7033		
Accuracy	0.8084			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	18,891	1,920	20,811	0.9077
Ans=1	3,310	7,879	11,189	0.7042
Total	22,201	9,799	32,000	
Precision	0.8509	0.8041		
F1 score	0.8784	0.7508		
Accuracy	0.8366			

0.5

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	18,617	2,531	21,148	0.8803
Ans=1	6,609	4,243	10,852	0.391
Total	25,226	6,774	32,000	
Precision	0.738	0.6264		
F1 score	0.8029	0.4814		
Accuracy	0.7144			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	18,507	2,607	21,114	0.8765
Ans=1	5,077	5,809	10,886	0.5336
Total	23,584	8,416	32,000	
Precision	0.7847	0.6902		
F1 score	0.8281	0.6019		
Accuracy	0.7599			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	18,985	2,276	21,261	0.8929
Ans=1	4,365	6,374	10,739	0.5935
Total	23,350	8,650	32,000	
Precision	0.8131	0.7369		
F1 score	0.8511	0.6575		
Accuracy	0.7925			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	19,629	1,789	21,418	0.9165
Ans=1	3,784	6,798	10,582	0.6424
Total	23,413	8,587	32,000	
Precision	0.8384	0.7917		
F1 score	0.8757	0.7093		
Accuracy	0.8258			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	19,767	1,841	21,608	0.9148
Ans=1	3,125	7,267	10392	0.6993
Total	22,892	9,108	32,000	
Precision	0.8635	0.7979		
F1 score	0.8884	0.7453		
Accuracy	0.8448			

0.6

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	19,768	1,766	21,534	0.918
Ans=1	7,375	3,091	10,466	0.2953
Total	27,143	4,857	32,000	
Precision	0.7283	0.6364		
F1 score	0.8122	0.4034		
Accuracy	0.7143			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	18,949	2,655	21,604	0.8771
Ans=1	5,315	5,081	10,396	0.4887
Total	24,264	7,736	32,000	
Precision	0.781	0.6568		
F1 score	0.8262	0.5604		
Accuracy	0.7509			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	19,315	2,492	21,807	0.8857
Ans=1	4,724	5,469	10,193	0.5365
Total	24,039	7,961	32,000	
Precision	0.8035	0.687		
F1 score	0.8426	0.6025		
Accuracy	0.7745			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	20,302	1,724	22,026	0.9217
Ans=1	3,703	6,271	9,974	0.6287
Total	24,005	7,995	32,000	
Precision	0.8457	0.7844		
F1 score	0.8821	0.698		
Accuracy	0.8304			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	20,958	1,359	22,317	0.9391
Ans=1	3,621	6,062	9,683	0.626
Total	24,579	7,421	32,000	
Precision	0.8527	0.8169		
F1 score	0.8938	0.7088		
Accuracy	0.8444			

0.7

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	20,678	1,346	22,024	0.9389
Ans=1	7,311	2,665	9,976	0.2671
Total	27,989	4,011	32,000	
Precision	0.7388	0.6644		
F1 score	0.8269	0.3811		
Accuracy	0.7295			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	19,789	2,391	22,180	0.8922
Ans=1	5,177	4,643	9,820	0.4728
Total	24,966	7,034	32,000	
Precision	0.7926	0.6601		
F1 score	0.8395	0.551		
Accuracy	0.7635			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	20,711	1,693	22,404	0.9244
Ans=1	4,619	4,977	9,596	0.5187
Total	25,330	6,670	32,000	
Precision	0.8176	0.7462		
F1 score	0.8678	0.612		
Accuracy	0.8027			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	20,217	2,448	22,665	0.892
Ans=1	3,503	5,832	9,335	0.6247
Total	23,720	8,280	32,000	
Precision	0.8523	0.7043		
F1 score	0.8717	0.6622		
Accuracy	0.814			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	21,223	1,864	23,087	0.9193
Ans=1	3,156	5,757	8,913	0.6459
Total	24,379	7,621	32,000	
Precision	0.8705	0.7554		
F1 score	0.8942	0.6964		
Accuracy	0.8431			

0.8

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	21,310	1,364	22,674	0.9398
Ans=1	7,072	2,254	9,326	0.2417
Total	28,382	3,618	32,000	
Precision	0.7508	0.623		
F1 score	0.8348	0.3483		
Accuracy	0.7364			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	21,072	1,890	22,962	0.9177
Ans=1	5,666	3,372	9,038	0.3731
Total	26,738	5,262	32,000	
Precision	0.7881	0.6408		
F1 score	0.848	0.4716		
Accuracy	0.7639			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	21,369	1,938	23,307	0.9168
Ans=1	4,480	4,213	8,693	0.4846
Total	25,849	6,151	32,000	
Precision	0.8267	0.6849		
F1 score	0.8694	0.5676		
Accuracy	0.7994			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	22,630	1,524	24,154	0.9369
Ans=1	3,442	4,404	7,846	0.5613
Total	26,072	5,928	32,000	
Precision	0.868	0.7429		
F1 score	0.9011	0.6395		
Accuracy	0.8448			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	22,630	1,524	24,154	0.9369
Ans=1	3,442	4,404	7,846	0.5613
Total	26,072	5,928	32,000	
Precision	0.868	0.7429		
F1 score	0.9011	0.6395		
Accuracy	0.8448			

0.9

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	22,047	1,491	23,538	0.9367
Ans=1	6,538	1,924	8,462	0.2274
Total	28,585	3,415	32,000	
Precision	0.7713	0.5634		
F1 score	0.846	0.324		
Accuracy	0.7491			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	22,950	1,345	24,295	0.9446
Ans=1	5,848	1,857	7,705	0.241
Total	28,798	3,202	32,000	
Precision	0.7969	0.58		
F1 score	0.8645	0.3405		
Accuracy	0.7752			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	23,183	1,728	24,911	0.9306
Ans=1	4,820	2,269	7,,089	0.3201
Total	28,003	3,997	32000	
Precision	0.8279	0.5677		
F1 score	0.8763	0.4093		
Accuracy	0.7954			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	24,022	1,513	25,535	0.9407
Ans=1	4,415	2,050	6,465	0.3171
Total	28,437	3,563	32,000	
Precision	0.8447	0.5754		
F1 score	0.8902	0.4089		
Accuracy	0.8147			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	24,469	1,798	26,267	0.9315
Ans=1	3,449	2,284	5,733	0.3984
Total	27,918	4,082	32,000	
Precision	0.8765	0.5595		
F1 score	0.9032	0.4654		
Accuracy	0.836			

Supplementary Table 3

Pred = “Prediction” : 0 = “unstained” , 1 = “stained”
Ans = “Answer” : 0 = “unstained” , 1 = “stained”

Input block size (px)

Target block size: 32 x 32 (px)

32 x 32

	Pred=0	Pred=1	Total	Recall
Ans=0	17,680	2,705	20,385	0.8673
Ans=1	6,259	5,356	11,615	0.4611
Total	23,939	8,061	32,000	
Precision	0.7385	0.6644		
F1 score	0.7978	0.5444		
Accuracy	0.7199			

64 x 64

	Pred=0	Pred=1	Total	Recall
Ans=0	17,991	2,394	20,385	0.8826
Ans=1	5,533	6,082	11,615	0.5236
Total	23,524	8,476	32,000	
Precision	0.7648	0.7176		
F1 score	0.8195	0.6054		
Accuracy	0.7523			

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	17,781	2,604	20,385	0.8723
Ans=1	4,420	7,195	11,615	0.6195
Total	22,201	9,799	32,000	
Precision	0.8009	0.7343		
F1 score	0.8351	0.672		
Accuracy	0.7805			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	17,888	2,497	20,385	0.8775
Ans=1	3,656	7,959	11,615	0.6852
Total	21,544	10,456	32,000	
Precision	0.8303	0.7612		
F1 score	0.8533	0.7212		
Accuracy	0.8077			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	18,397	1,988	20,385	0.9025
Ans=1	3,173	8,442	11,615	0.7268
Total	21,570	10,430	32,000	
Precision	0.8529	0.8094		
F1 score	0.877	0.7659		
Accuracy	0.8387			

Target block size: 128 x 128 (px)

128 x 128

	Pred=0	Pred=1	Total	Recall
Ans=0	18,409	2,852	21,261	0.8659
Ans=1	3,388	7,351	10,739	0.6845
Total	21,797	10,203	32,000	
Precision	0.8446	0.7205		
F1 score	0.8551	0.702		
Accuracy	0.805			

256 x 256

	Pred=0	Pred=1	Total	Recall
Ans=0	18,187	1,873	20,060	0.9066
Ans=1	3,946	7,994	11,940	0.6695
Total	22,133	9,867	32,000	
Precision	0.8217	0.8102		
F1 score	0.8621	0.7332		
Accuracy	0.8182			

512 x 512

	Pred=0	Pred=1	Total	Recall
Ans=0	18,356	1,704	20,060	0.9151
Ans=1	3,645	8,295	11,940	0.6947
Total	22,001	9,999	32,000	
Precision	0.8343	0.8296		
F1 score	0.8728	0.7562		
Accuracy	0.8328			

Supplementary Table 4

Pred = “Prediction” : 0 = “unstained” , 1 = “stained”
Ans= “Answer” : 0 = “unstained” , 1 = “stained”

A

Small network

Automatically binarized

	Pred=0	Pred=1	Total	Recall
Ans=0	18,043	1,783	19,826	0.9101
Ans=1	2,724	9,450	12,174	0.7762
Total	20,767	11,233	32,000	
Precision	0.8688	0.8413		
F1 score	0.889	0.8075		
Accuracy	0.8592			

Large network

Automatically binarized

	Pred=0	Pred=1	Total	Recall
Ans=0	18,291	1,535	19,826	0.9226
Ans=1	1,574	10,600	12,174	0.8707
total	19,865	12,135	32,000	
Precision	0.9208	0.8735		
F1 score	0.9217	0.8721		
Accuracy	0.9028			

Small network

Rebinarized

	Pred=0	Pred=1	Total	Recall
Ans=0	23,556	974	24,530	0.9603
Ans=1	1,354	6,116	7,470	0.8187
Total	24,910	7,090	32,000	
Precision	0.9456	0.8626		
F1 score	0.9529	0.8401		
Accuracy	0.9273			

Large network

Rebinarized

	Pred=0	Pred=1	Total	Recall
Ans=0	23,927	603	24,530	0.9754
Ans=1	622	6,848	7,470	0.9167
Total	24,549	7,451	32,000	
Precision	0.9747	0.9191		
F1 score	0.975	0.9179		
Accuracy	0.9617			

B

Small network
Automatically binarized

Fold 1

	Pred=0	Pred=1	Total	Recall
Ans=0	17,764	12,928	30,692	0.5788
Ans=1	488	8,820	9,308	0.9476
Total	18,252	21,748	40,000	
Precision	0.9733	0.4056		
F1 score	0.7259	0.568		
Accuracy	0.6646			

Large network
Rebinarized

	Pred=0	Pred=1	Total	Recall
Ans=0	34,853	2,740	37,593	0.9271
Ans=1	972	1,435	2,407	0.5962
Total	35,825	4,175	40,000	
Precision	0.9729	0.3437		
F1 score	0.9494	0.436		
Accuracy	0.9072			

Fold 2

	Pred=0	Pred=1	Total	Recall
Ans=0	14,204	3,980	18,184	0.7811
Ans=1	3,679	18,137	21,816	0.8314
Total	17,883	22,117	40,000	
Precision	0.7943	0.82		
F1 score	0.7876	0.8257		
Accuracy	0.8085			

	Pred=0	Pred=1	Total	Recall
Ans=0	18,096	1,505	19,601	0.9232
Ans=1	3,984	16,415	20,399	0.8047
Total	22,080	17,920	40,000	
Precision	0.8196	0.916		
F1 score	0.8683	0.8568		
Accuracy	0.8628			

Fold 3

	Pred=0	Pred=1	Total	Recall
Ans=0	20,037	1,934	21,971	0.912
Ans=1	6,803	11,226	18,029	0.6227
Total	26,840	13,160	40,000	
Precision	0.7465	0.853		
F1 score	0.821	0.7199		
Accuracy	0.7816			

	Pred=0	Pred=1	Total	Recall
Ans=0	28,847	749	29,596	0.9747
Ans=1	2,245	8,159	10,404	0.7842
Total	31,092	8,908	40,000	
Precision	0.9278	0.9159		
F1 score	0.9507	0.845		
Accuracy	0.9252			

Fold 4

	Pred=0	Pred=1	Total	Recall
Ans=0	23,713	5,601	29,314	0.8089
Ans=1	3,116	7,570	10,686	0.7084
Total	26,829	13,171	40,000	
Precision	0.8839	0.5747		
F1 score	0.8447	0.6346		
Accuracy	0.7821			

	Pred=0	Pred=1	Total	Recall
Ans=0	34,920	952	35,872	0.9735
Ans=1	734	3,394	4,128	0.8222
Total	35,654	4,346	40,000	
Precision	0.9794	0.7809		
F1 score	0.9764	0.801		
Accuracy	0.9578			