Electronic Supplementary Material (ESI) for Digital Discovery.

Supporting information
for the article

Digital biology approach for macroscale studies of biofilm growth and biocide effects with electron microscopy

by

Konstantin S. Kozlov,[a,c,§] Daniil A. Boiko,[a,§] Elena V. Detusheva,[b] Konstantin V. Detushev,[b] Evgeniy O. Pentsak,[a] Anatoly N. Vereshchagin,[a] Valentine P. Ananikov[a,c,*]

[a] Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospekt 47, Moscow, 119991, Russia
[b] State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow region, Russia
[c] Lomonosov Moscow State University, Chemistry Department, Leninskie Gory 1/3, Moscow, 119991, Russia

[§] These authors contributed equally
* E-mail: val@ioc.ac.ru

Contents

1 Neural network

Additional information about the neural network and training.

1.1 Architecture

We used **U-Net**[1] a convolutional neural network architecture that is actively used in the segmentation of biomedical images. The network consists of the encoding and decoding parts connected with skip connections. The encoder used in the research was **ResNet34**.[2] The main advantage of U-Net is that it can be trained with few images.

1.2 Optimizer

We used the **Adam optimizer**,[3] one of the most widely used optimization algorithms in deep learning. The algorithm can be described as follows:

1. Accumulation of the vector of inertia using a moving average

$$m_i^{(t+1)} = \alpha m_i^{(t)} + (1-\alpha)\nabla_i L(w^{(t)})$$

2. Accumulation of the normalization factor of the gradient step using the moving average

$$v_i^{(t+1)} = \beta v_i^{(t)} + (1-\beta)(\nabla_i L(w^t))^2$$

3. Bias correction

$$\hat{m}_i^{(t+1)} = \frac{m_i^{(t+1)}}{1-\alpha^t}$$

$$\hat{v}_i^{(t+1)} = \frac{v_i^{(t+1)}}{1-\beta^t}$$

4. Weight recalculation

$$w_i^{(t+1)} = w_i^{(t)} - \frac{\eta}{\sqrt{\hat{v}_i^{(t+1)} + \varepsilon}}\hat{m}_i^{(t+1)}$$

1.3 Loss functions

As described in the “Methods” section, we used a linear combination of **Binary Cross Entropy** and **Dice coefficient** as our loss function. Mathematical formulas are shown below:

$$BCE(y,\hat{p}) = -y\log\hat{p} + (1-y)\log(1-\hat{p})$$

$$Dice(y,\hat{p}) = 1 - \frac{2y\hat{p}+1}{y+\hat{p}+1}$$

2 Additional model training details

2.1 Loss curve

Here, we report the validation loss and IOU curves of the final network.

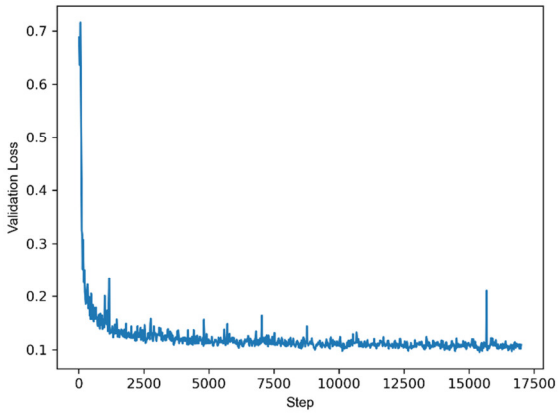

Figure 1. Validation loss.

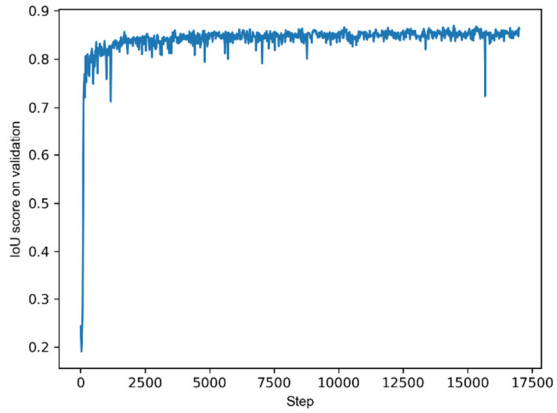

Figure 2. Intersection-over-Union Score on validation samples.

2.2 Statistical significance of using a larger amount of training data

Here, we show validation IoU scores for the networks trained with the same hyperparameters but with different training data (the first group of models was trained only on 4 original images, the second group was trained on 4 original images and 4 elastic transforms, and the third group was trained on 72 original images with 9 elastic transforms).

Table 1. Validation IoU scores of networks trained on different amounts of data

Sample	**4** original + **0** elastic transformed (group 1)	**4** original + **4** elastic transformed (group 2)	**72** original + **9** elastic transformed (group 3)
1	0.8145	0.803	0.8511
2	0.8153	0.826	0.854
3	0.7786	0.8179	0.8515
4	0.7492	0.8184	0.86
Mean IoU	0.7894	0.8163	0.8542

Mann–Whitney U test for group 1 and group 2:
p-value = 0.1143

Mann–Whitney U test for group 2 and group 3:
p-value = 0.02857

The results were obtained using the *wilcox.test* function in R language.

3 Model predictions

Here, we present neural network mapping and predictions for the images described in the article.

3.1 Model predictions on validation samples

Segmentation results of the final neural network on validation.

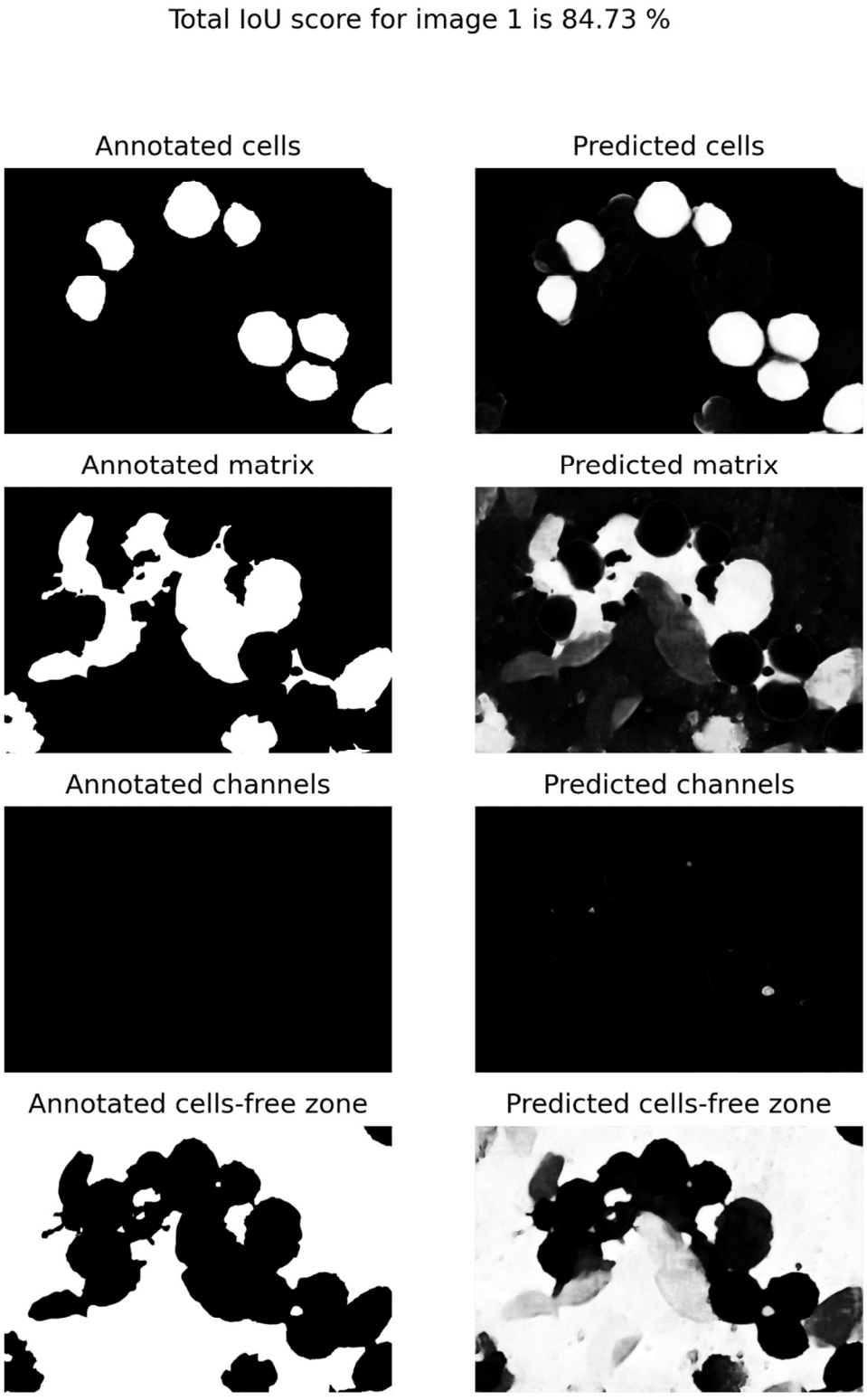

Figure 3. Model predictions on validation image 1.

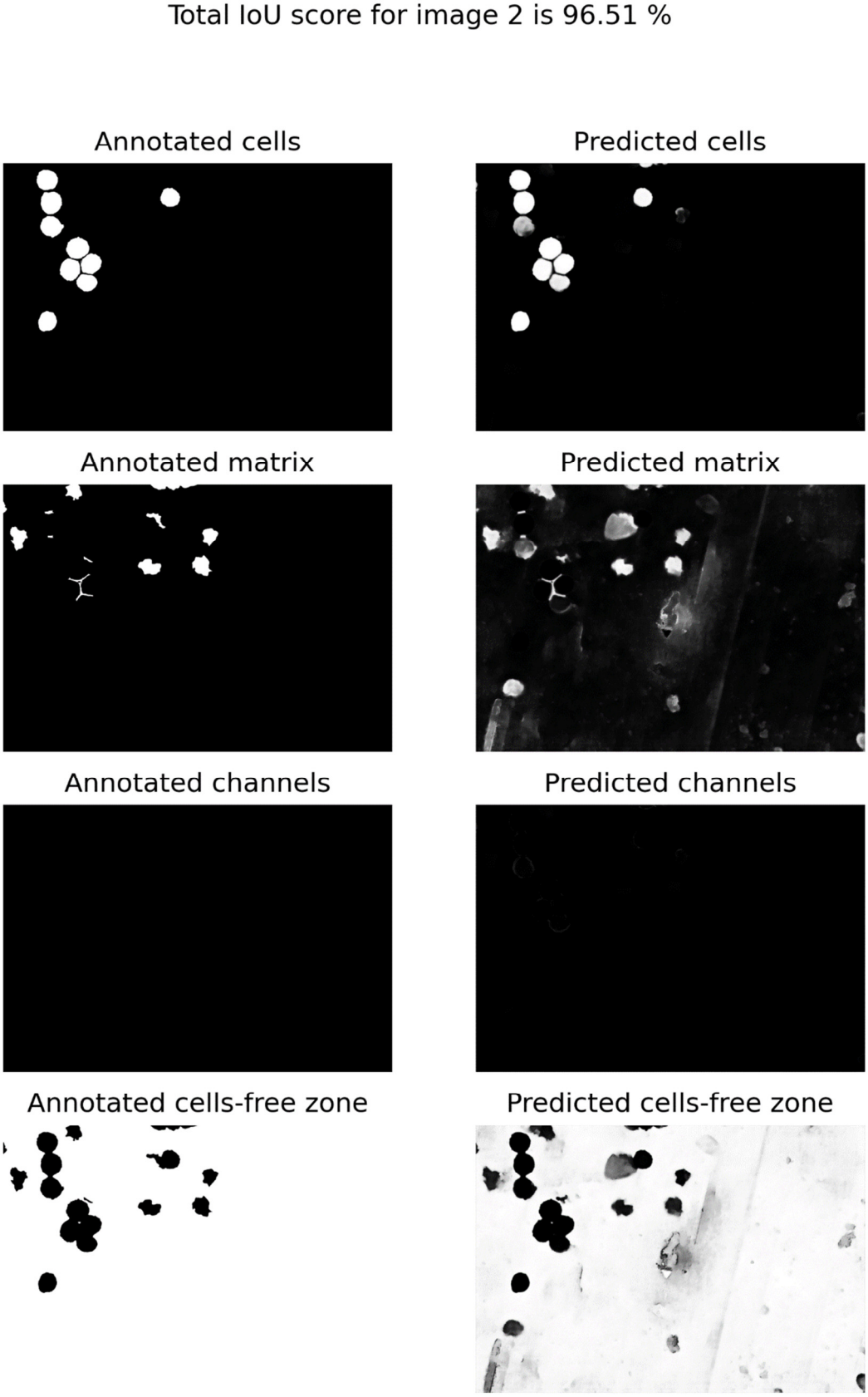

Figure 4. Model predictions on validation image 2.

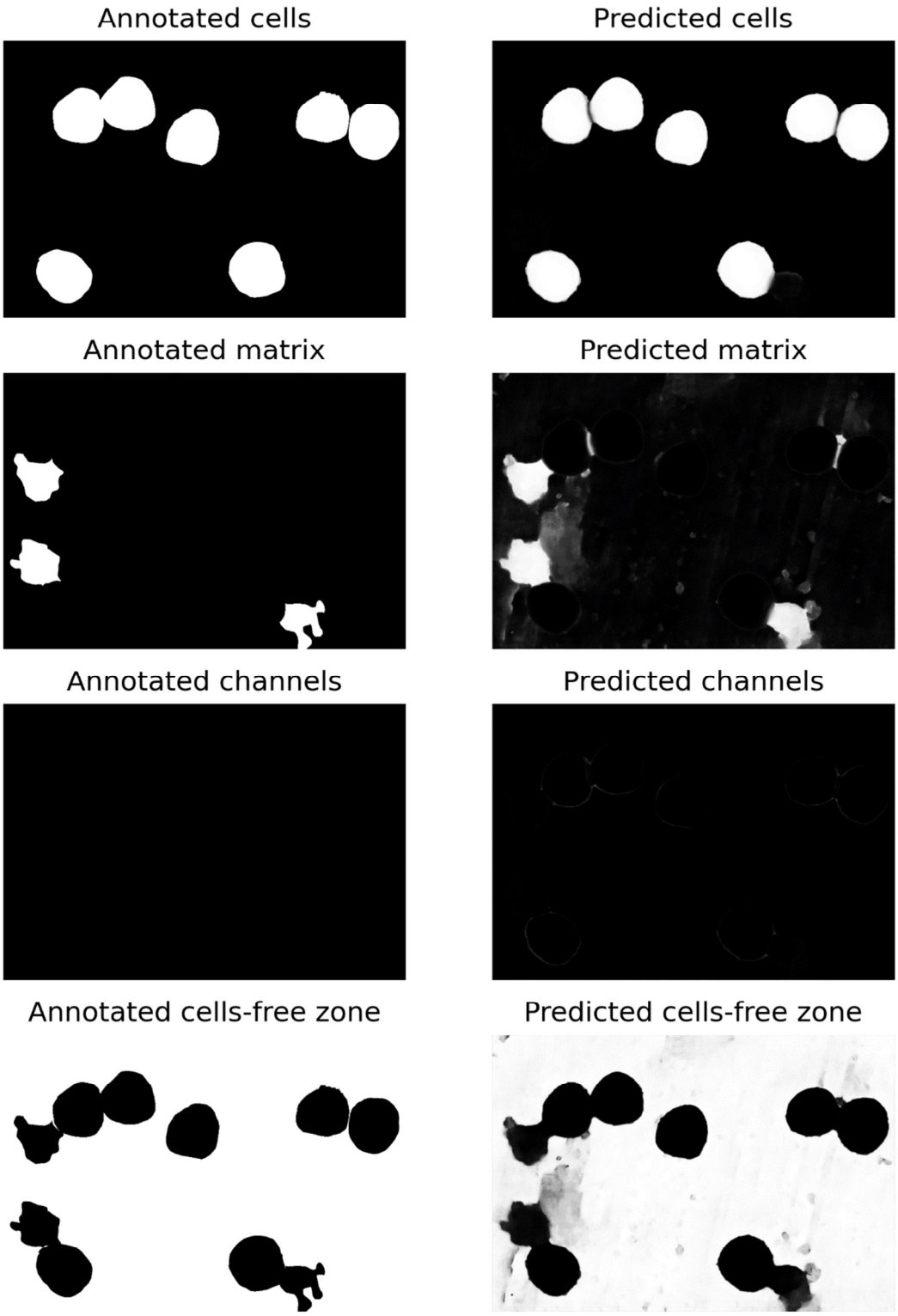

Figure 5. Model predictions on validation image 3.

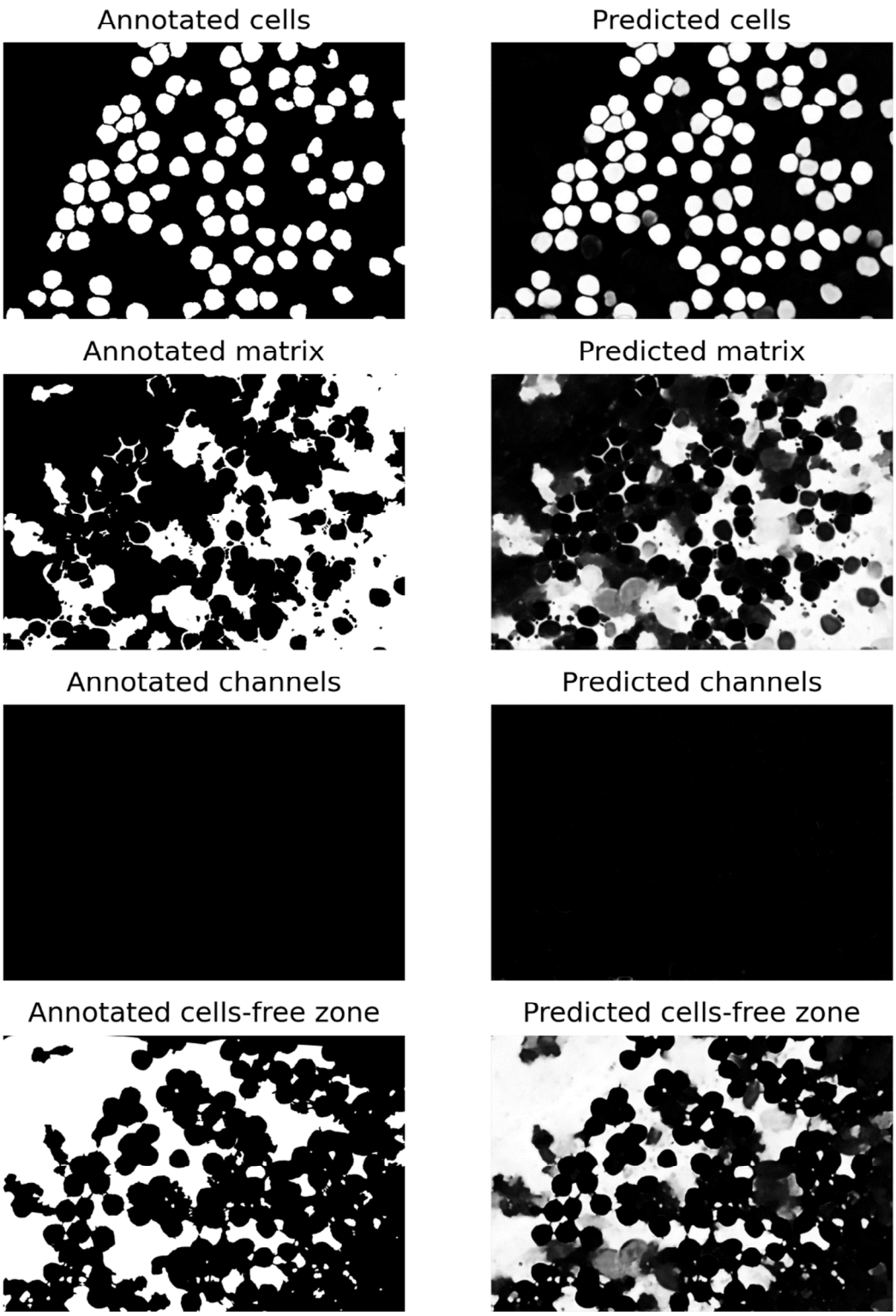

Figure 6. Model predictions on validation image 4.

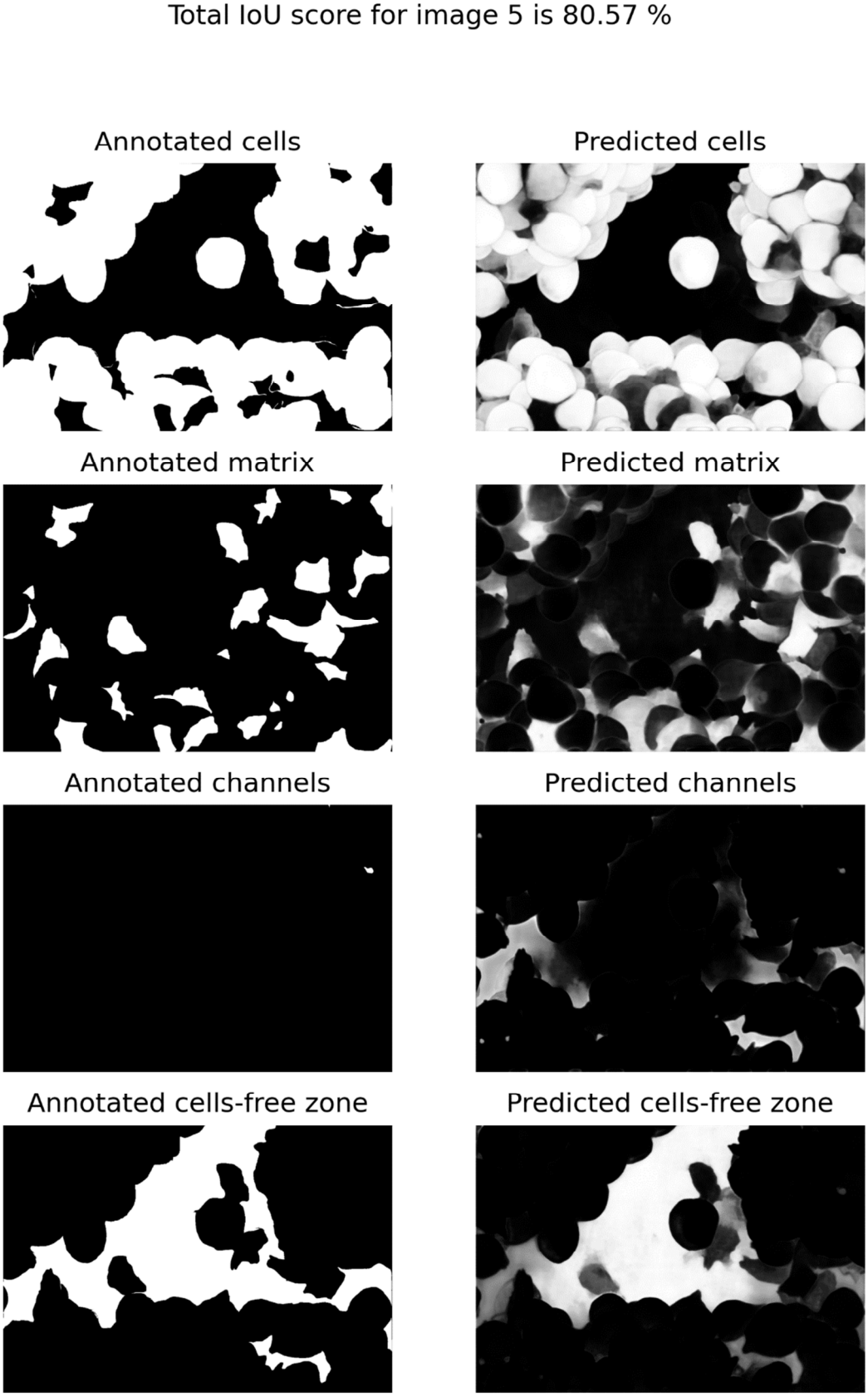

Figure 7. Model prediction on validation image 5.

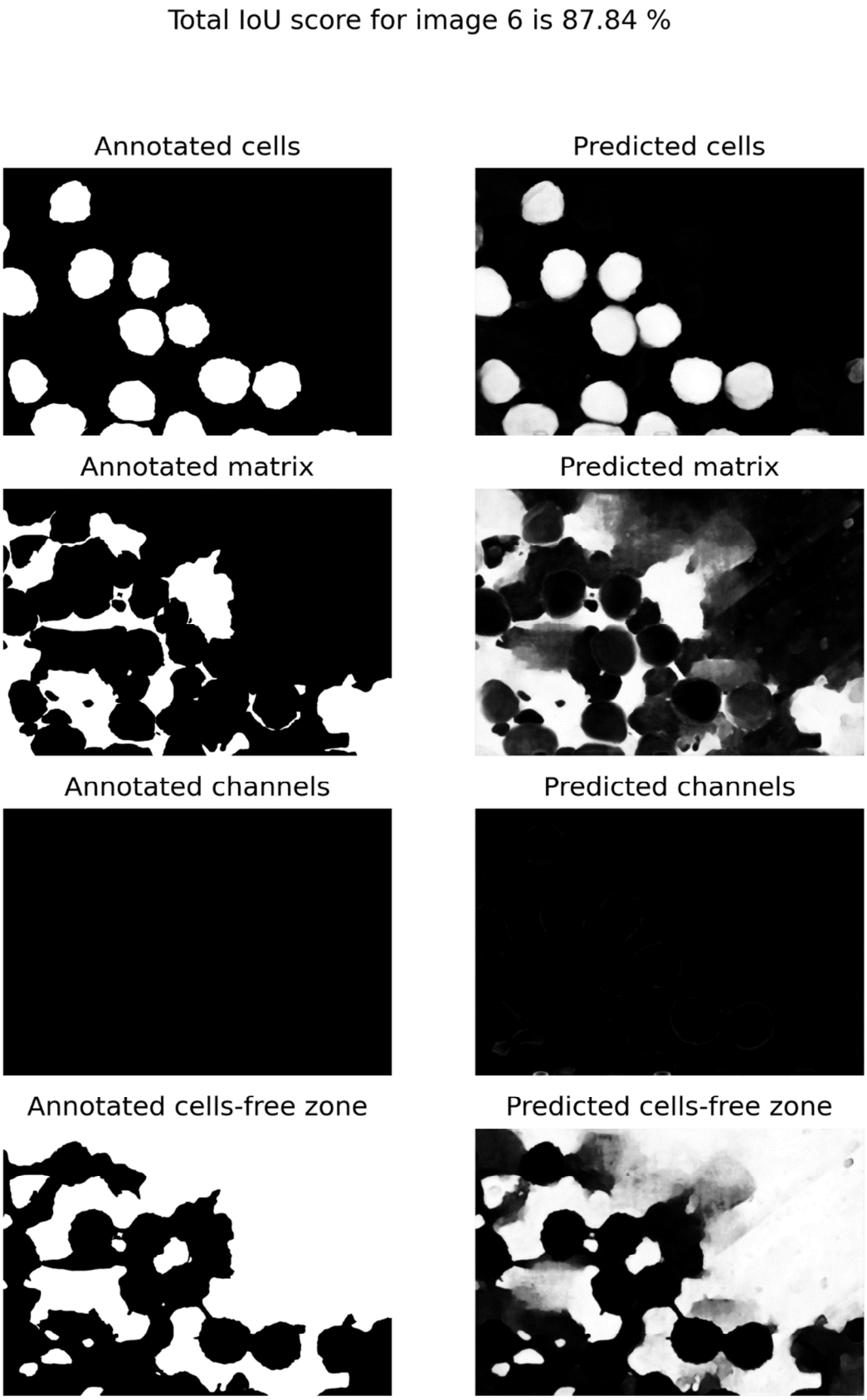

Figure 8. Model predictions on validation image 6.

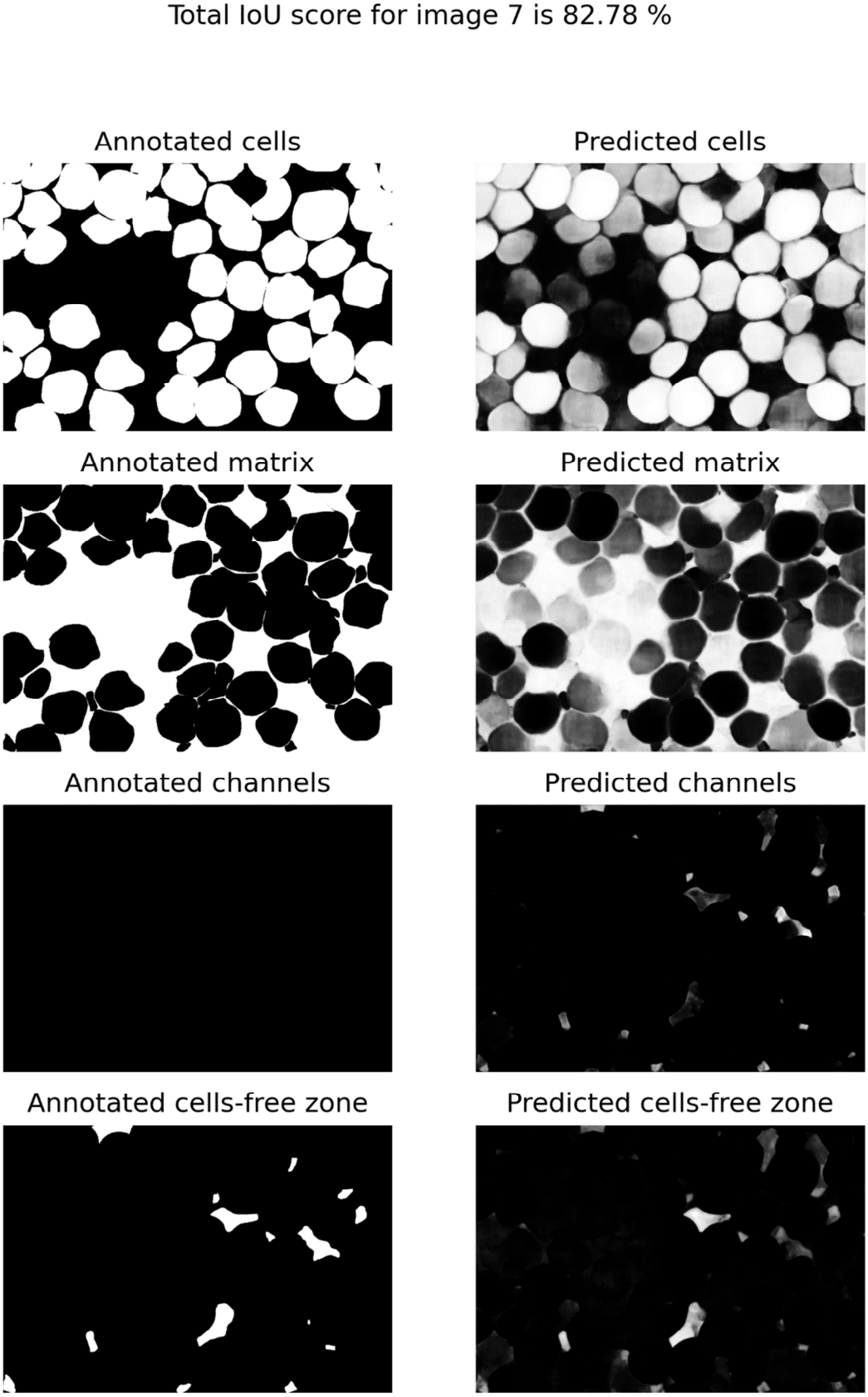

Figure 9. Model predictions on validation image 7.

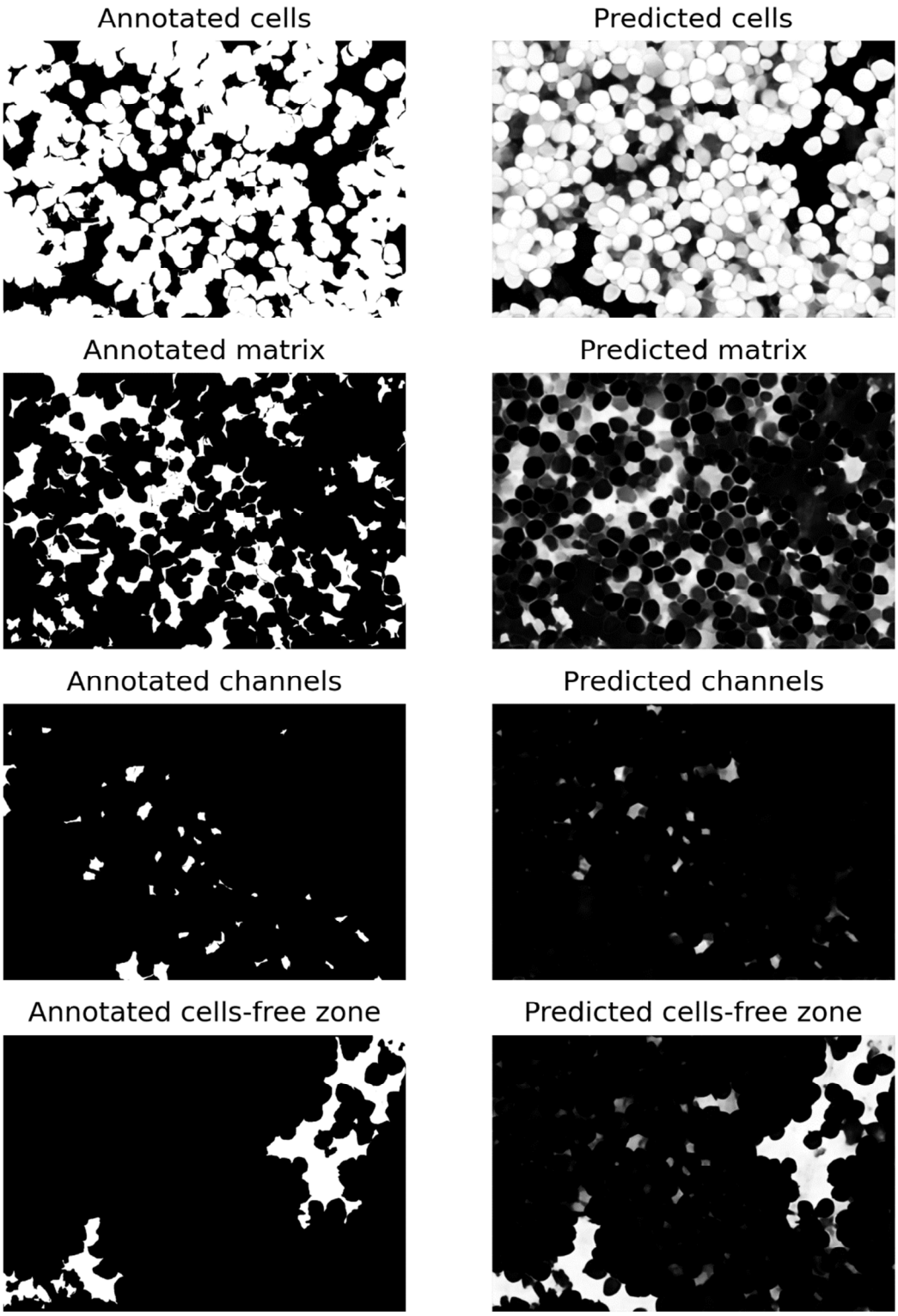

Figure 10. Model prediction on validation image 8.

3.2 Model predictions on test samples

Segmentation results of the final neural network on test.

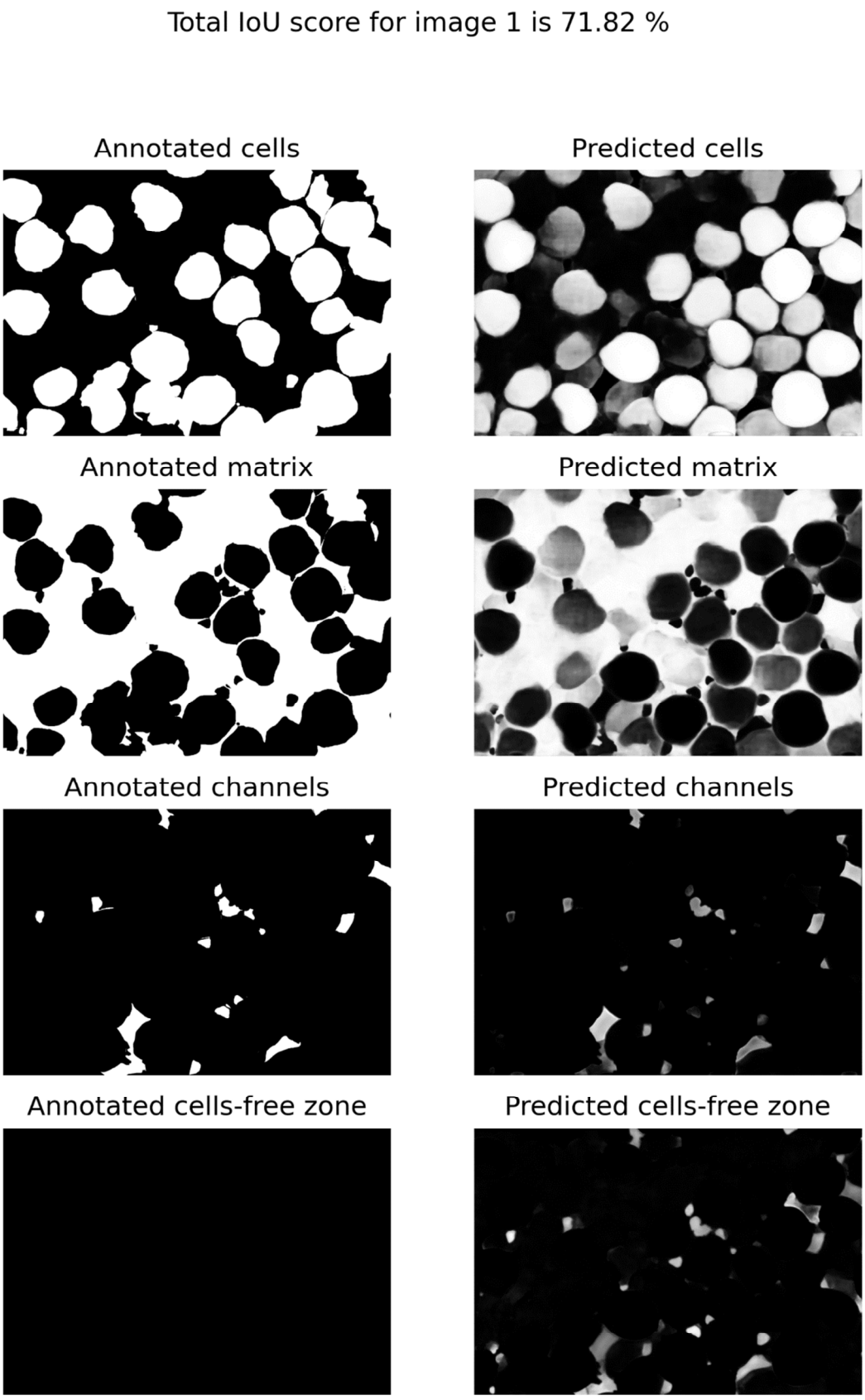

Figure 11. Model predictions on test image 1.

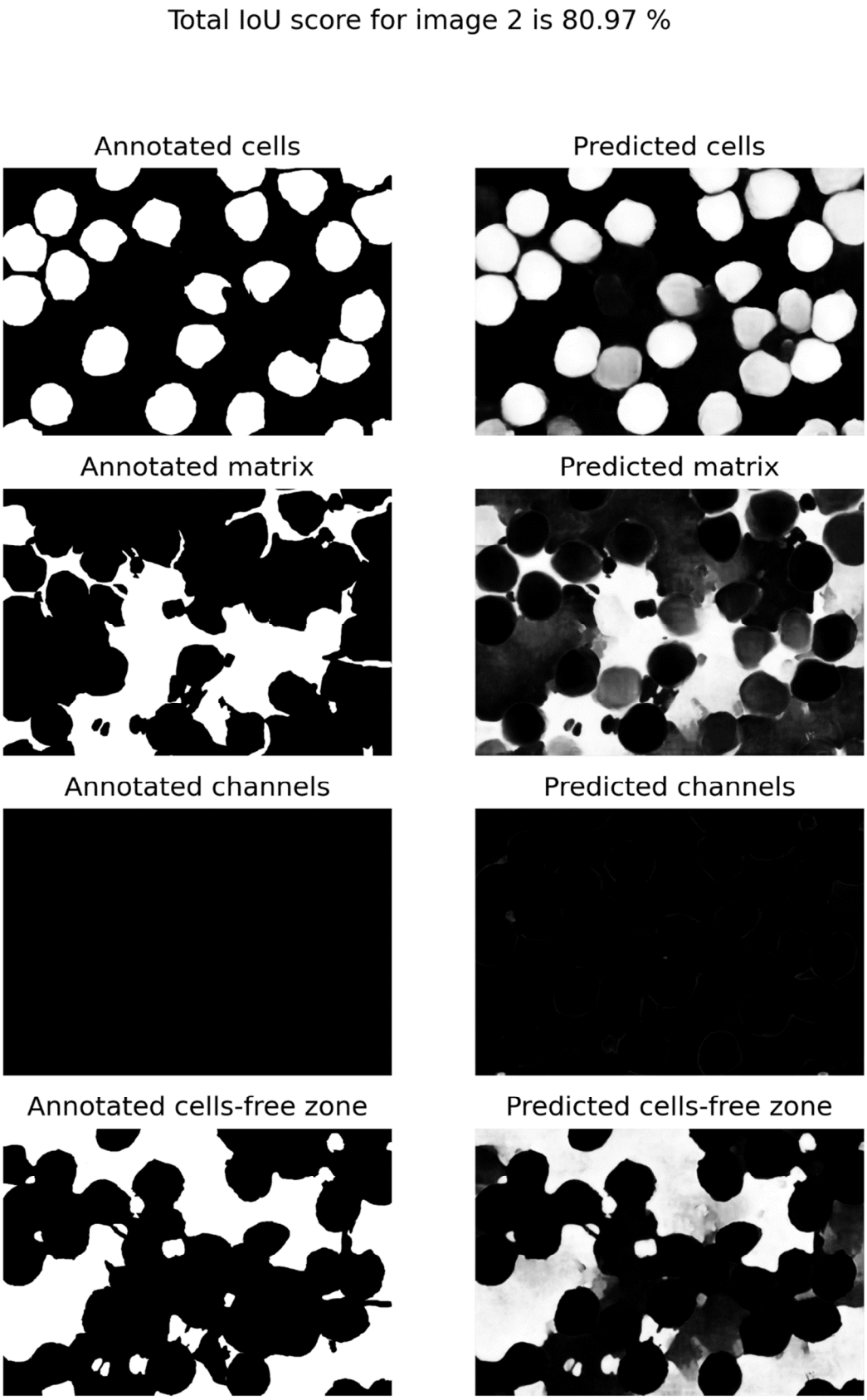

Figure 12. Model predictions on test image 2.

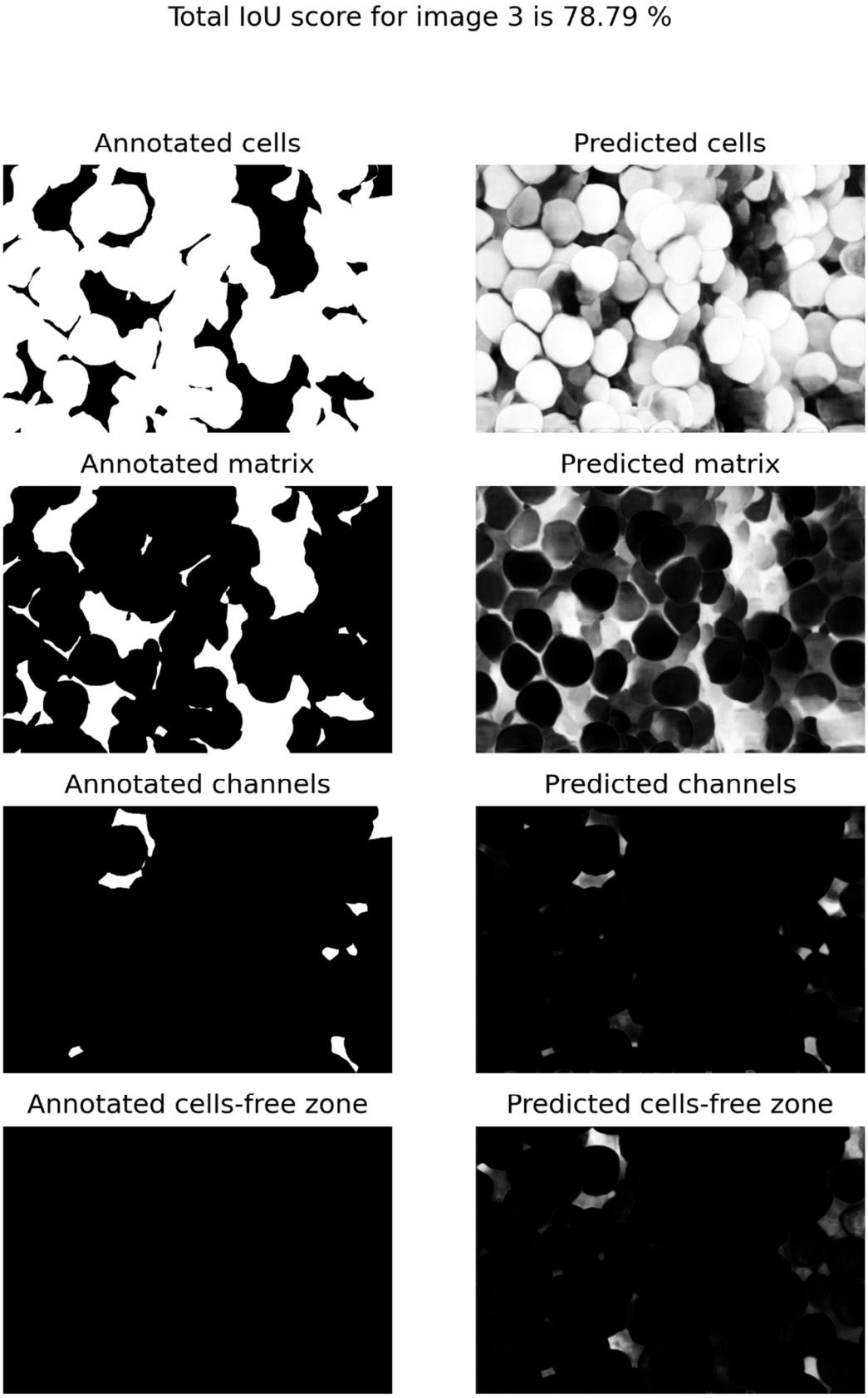

Figure 13. Model predictions on test image 3.

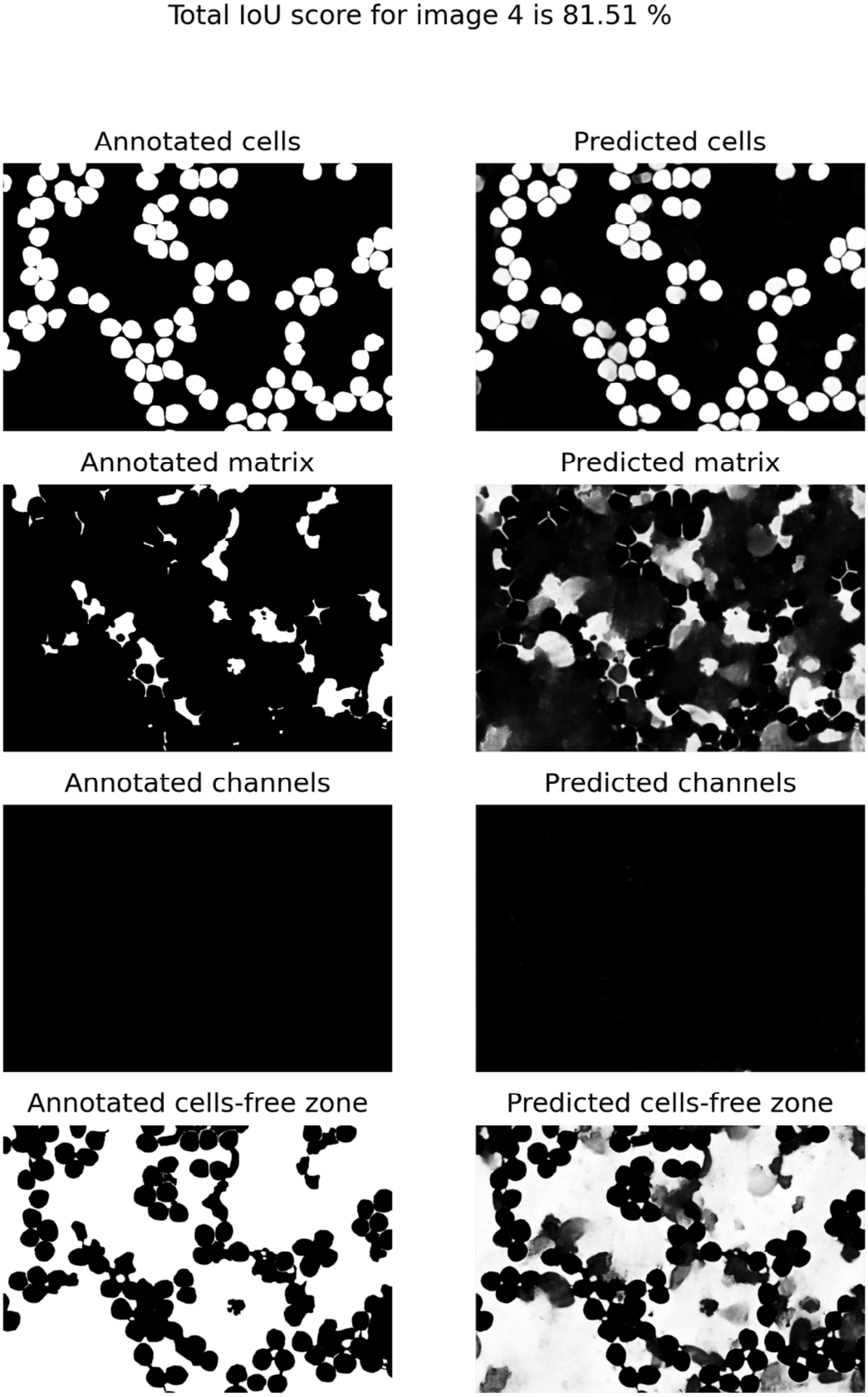

Figure 14. Model predictions on test image 3.

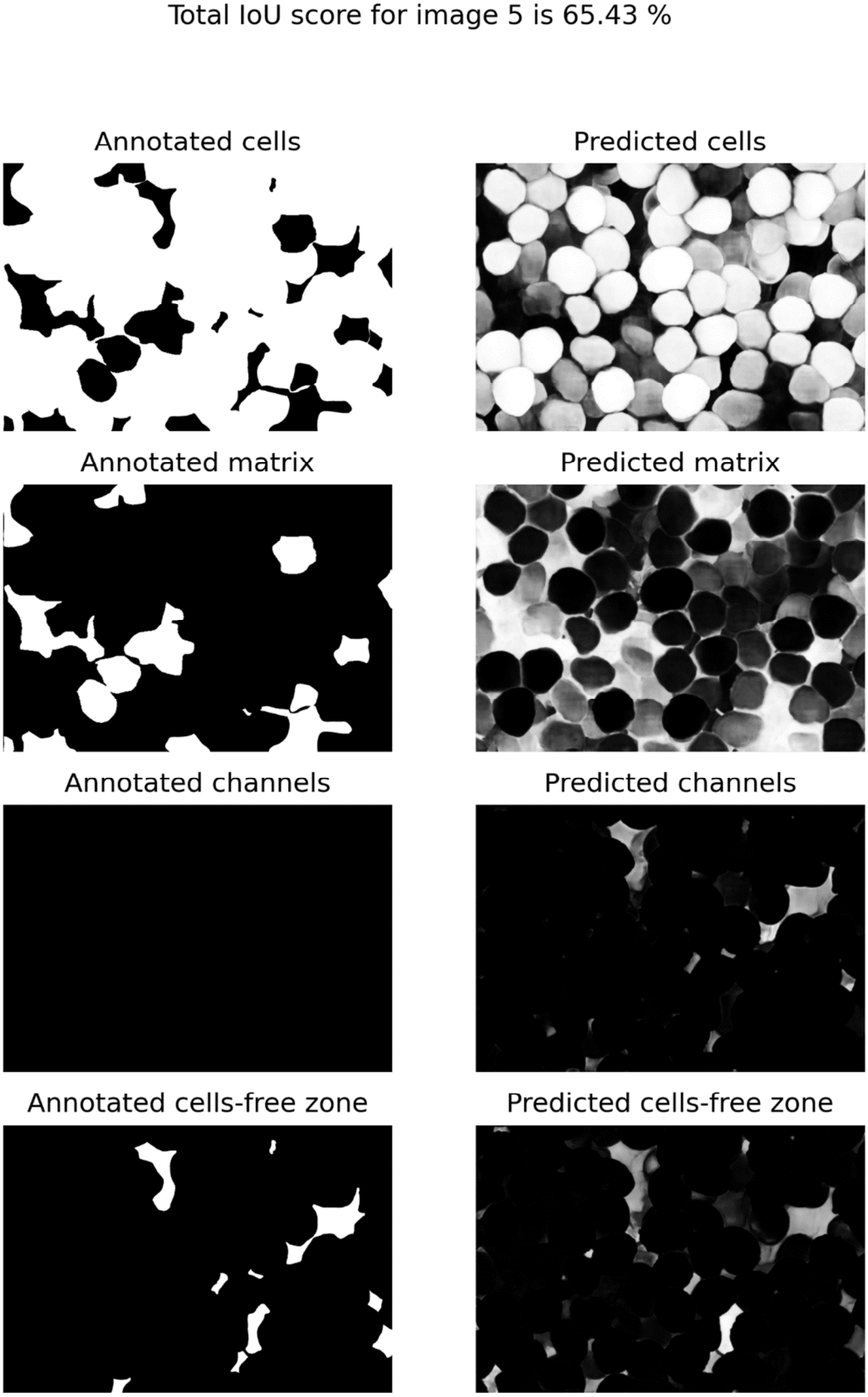

Figure 15. Model predictions on test image 5.

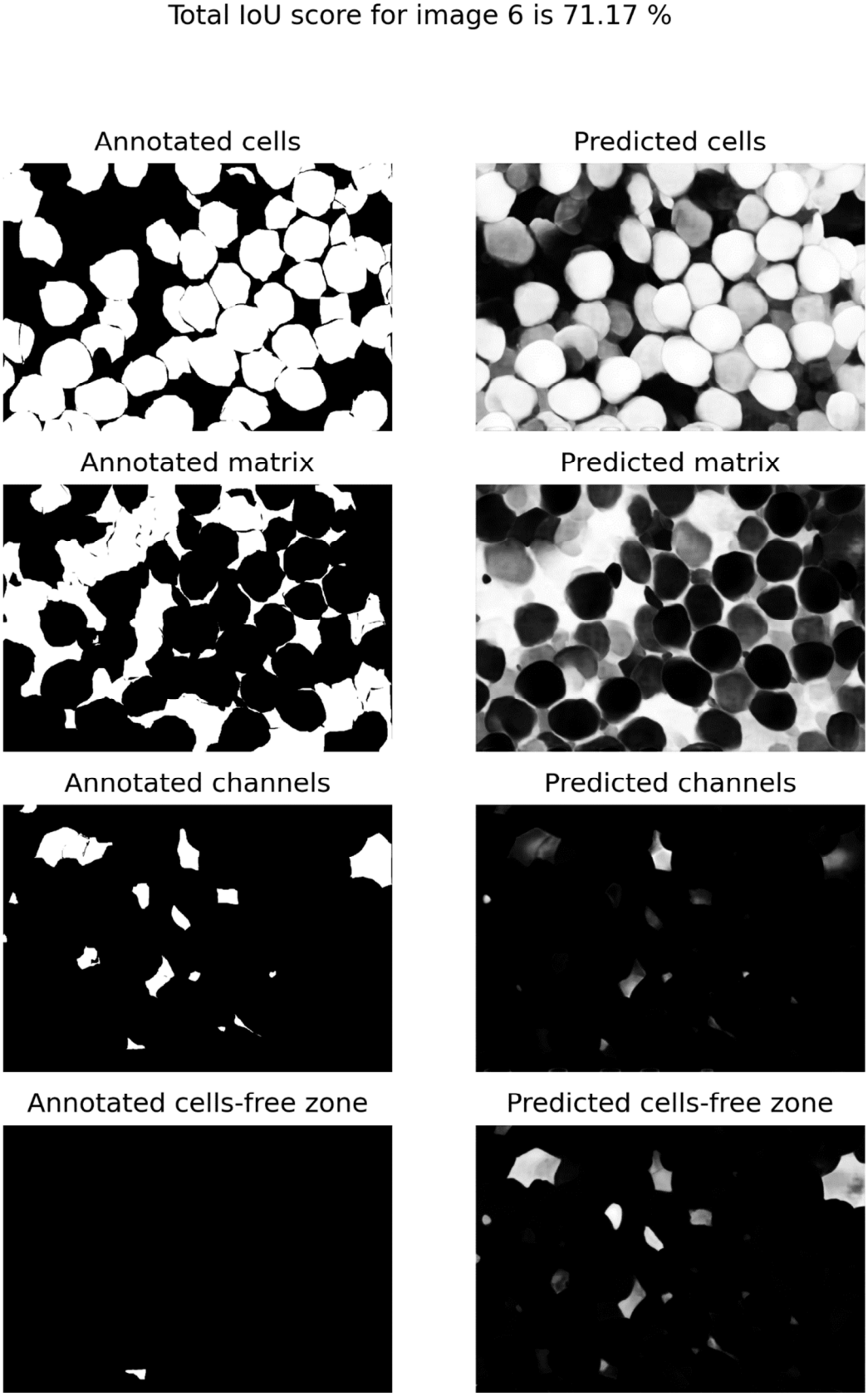

Figure 16. Model predictions on test image 6.

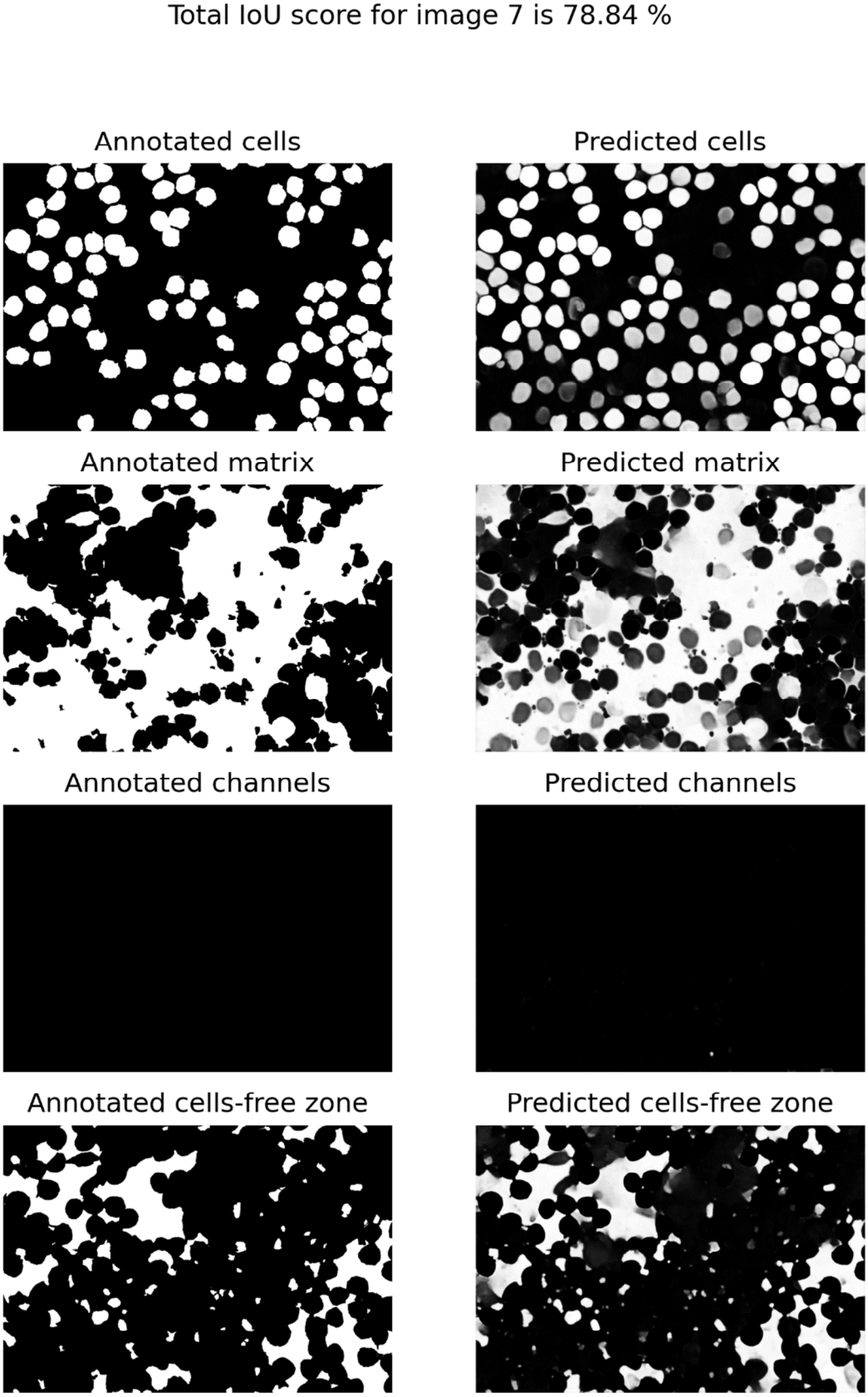

Figure 17. Model predictions on test image 7.

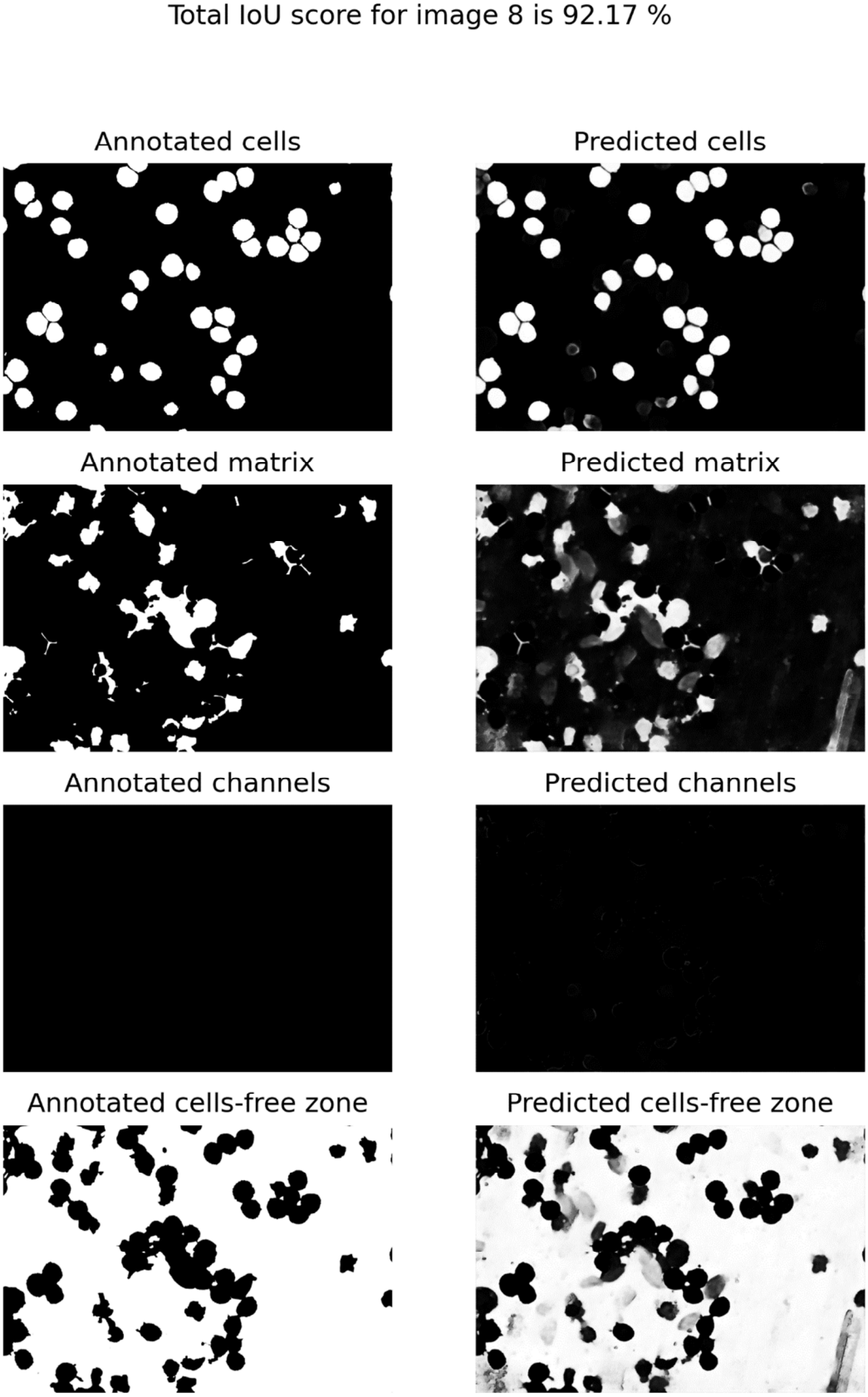

Figure 18. Model predictions on test image 8.

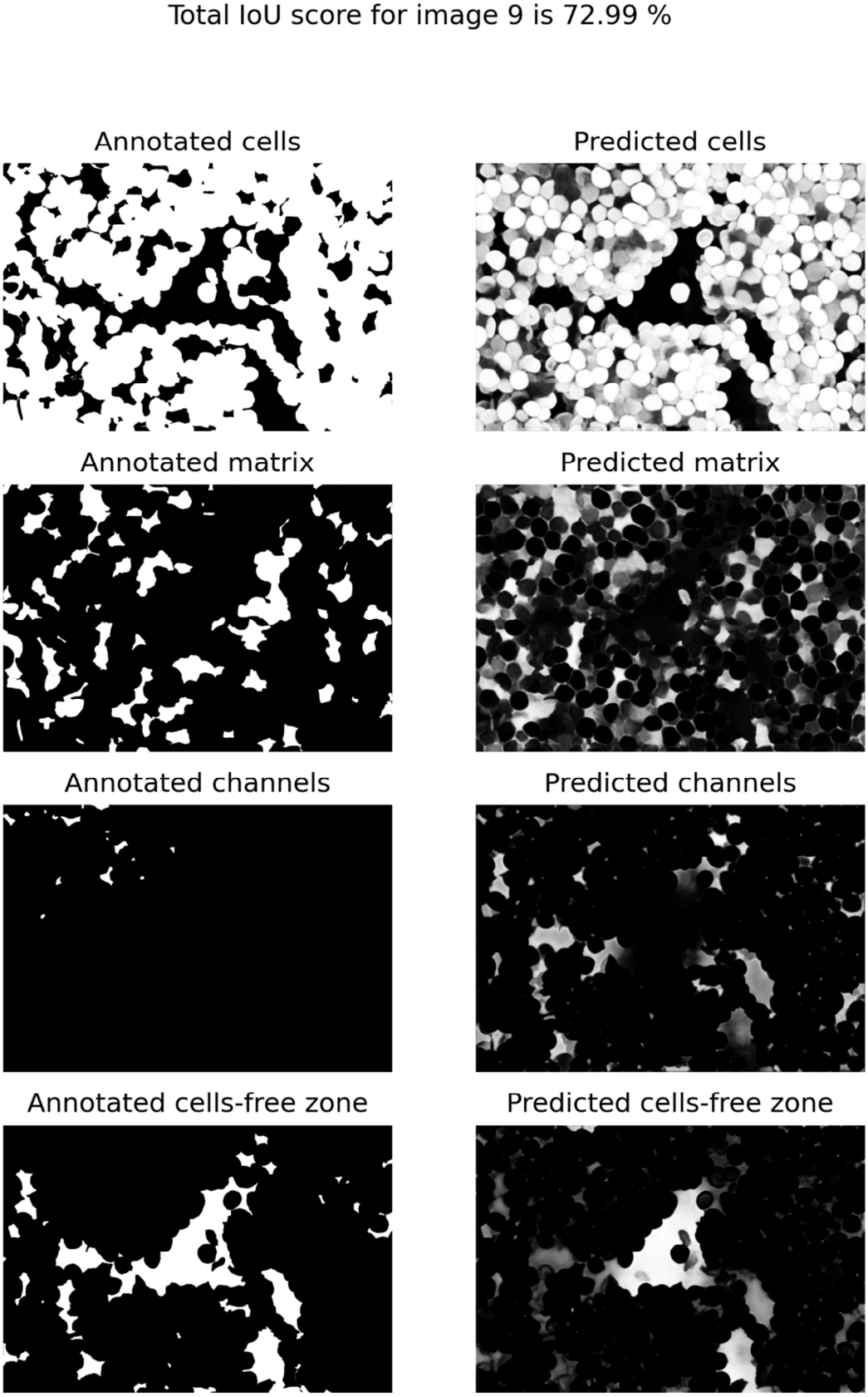

Figure 19. Model predictions on test image 9.

3.3 Neural network-based segmentation of cherry-picked examples

These segmentation maps refer to area analysis, which was discussed in the article in the “Object of study” section.

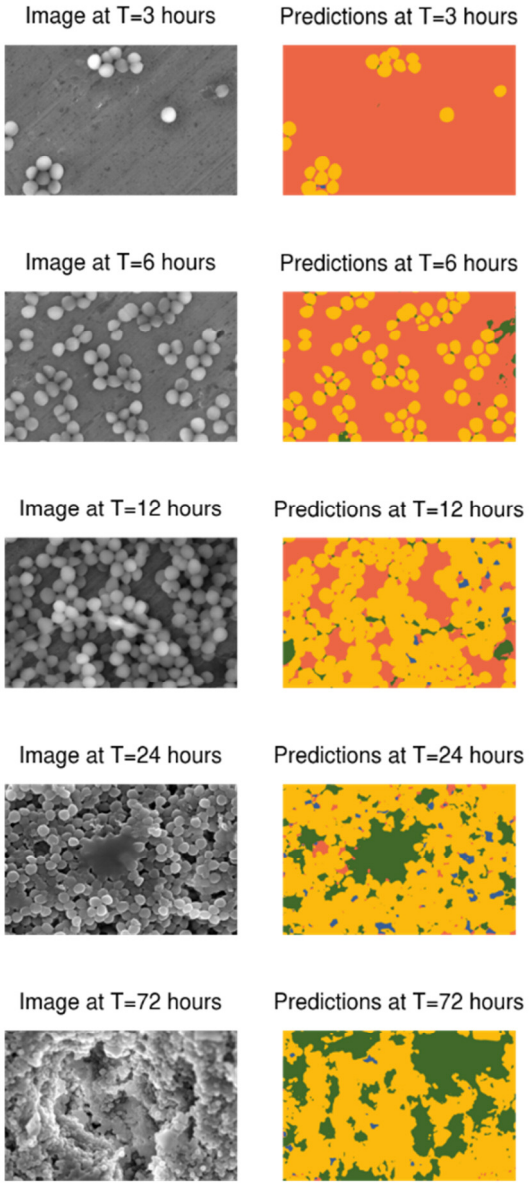

Figure 20. Neural network segmentation of cherry-picked images as an example of biofilm formation

3.4 Neural network-based predictions of a large number of SEM images for biofilm growth estimation

Here, we report statistics of segmentation class areas on each image, which were used for the calculation of biofilm formation rate constants.

Table 2. Area statistics for biofilm images after 1 hour.

Image number	Cells	Matrix	Channels	Cells-free zone
0	0.5%	0.7%	0%	98.8%
1	0.4%	0.4%	0%	99.2%

2	0.6%	0.9%	0%	98.4%
3	0.4%	0.5%	0%	99.0%
4	0.6%	1.0%	0%	98.4%
5	0.4%	0.4%	0%	99.3%
6	0.3%	0.4%	0%	99.3%
7	0.7%	0.7%	0%	98.6%
8	0.7%	0.4%	0%	98.9%
9	0.7%	0.9%	0%	98.5%
10	1.1%	0.7%	0%	98.1%
11	0.7%	0.5%	0%	98.8%
12	0.3%	0.4%	0%	99.3%
13	0.3%	0.7%	0%	99.0%
14	0.7%	1.2%	0%	98.2%
15	0.4%	0.5%	0%	99.1%
16	0.3%	0.7%	0%	98.9%
17	0.4%	0.5%	0%	99.1%
18	0.4%	0.4%	0%	99.3%
19	1.1%	1.2%	0%	97.7%
20	1.1%	1.1%	0%	97.9%
21	1.1%	0.8%	0%	98.1%
22	1.0%	1.2%	0%	97.8%
23	0.5%	0.8%	0%	98.7%
24	0.4%	0.5%	0%	99.1%
25	0.3%	0.6%	0%	99.0%
26	0.3%	0.4%	0%	99.3%
27	0.4%	1.1%	0%	98.5%
28	0.3%	0.3%	0%	99.4%
29	0.5%	0.8%	0%	98.7%
30	9.3%	12.3%	0%	78.4%
31	12.2%	33.7%	0%	54.1%
32	6.4%	18.8%	0%	74.8%
33	0.2%	0.5%	0%	99.3%
34	0.4%	0.8%	0%	98.8%
35	0.2%	0.4%	0%	99.3%
36	0.3%	0.6%	0%	99.0%
37	0.4%	0.3%	0%	99.3%
38	0.3%	0.5%	0%	99.2%
39	0.4%	0.5%	0%	99.2%
40	13.6%	42.6%	0%	43.8%
41	7.7%	34.0%	0%	58.3%
42	2.9%	19.1%	0%	78.0%
43	0.5%	1.1%	0%	98.4%
44	0.8%	0.8%	0%	98.4%
45	0.3%	0.8%	0%	98.9%
46	0.3%	0.8%	0%	98.9%
47	0.3%	0.7%	0%	99.0%
48	0.3%	0.3%	0%	99.4%
49	0.6%	0.8%	0%	98.6%

50	6.5%	1.3%	0%	92.1%
51	14.0%	45.5%	0%	40.5%
52	6.0%	19.9%	0%	74.1%
53	0.4%	0.5%	0%	99.0%
54	1.2%	0.6%	0%	98.2%
55	0.9%	0.3%	0%	98.8%
56	0.9%	1.0%	0%	98.2%
57	0.5%	0.6%	0%	98.9%
58	0.3%	0.5%	0%	99.3%
59	0.8%	0.8%	0%	98.4%
60	5.3%	1.0%	0%	93.7%
61	5.5%	2.0%	0%	92.4%
62	5.2%	1.0%	0%	93.8%
63	0.6%	0.5%	0%	98.9%
64	0.3%	0.9%	0%	98.8%
65	0.3%	0.5%	0%	99.3%
66	0.5%	1.0%	0%	98.5%
67	0.3%	1.1%	0%	98.6%
68	0.4%	0.5%	0%	99.1%
69	0.5%	0.7%	0%	98.8%
70	0.9%	0.7%	0%	98.3%
71	3.0%	1.2%	0%	95.8%
72	3.5%	0.4%	0%	96.1%
73	0.5%	0.9%	0%	98.7%
74	0.3%	1.0%	0%	98.7%
75	0.4%	0.7%	0%	98.9%
76	0.5%	0.5%	0%	99.0%
77	0.3%	0.6%	0%	99.1%
78	0.5%	1.0%	0%	98.5%
79	0.4%	0.8%	0%	98.9%
80	2.4%	10.8%	0%	86.8%
81	2.1%	1.0%	0%	97.0%
82	0.6%	1.3%	0%	98.0%
83	0.9%	0.6%	0%	98.5%
84	0.4%	0.9%	0%	98.7%
85	0.4%	0.7%	0%	98.9%
86	0.2%	0.8%	0%	99.0%
87	0.3%	0.3%	0%	99.4%
88	0.4%	0.8%	0%	98.8%
89	0.4%	1.1%	0%	98.5%
90	0.9%	0.6%	0%	98.5%
91	1.9%	2.2%	0%	95.9%
92	0.5%	0.4%	0%	99.0%
93	0.5%	0.9%	0%	98.6%
94	0.4%	1.1%	0%	98.5%
95	0.8%	1.1%	0%	98.1%
96	0.3%	1.1%	0%	98.6%
97	0.4%	0.9%	0%	98.7%

98	0.5%	0.5%	0%	99.0%
99	0.5%	0.6%	0%	98.9%

Table 3. Area statistics for biofilm images after 3 hours.

Image number	Cells	Matrix	Channels	Cells-free zone
0	10.5%	1.1%	0%	88.4%
1	13.3%	0.7%	0%	86.1%
2	9.3%	0.4%	0%	90.4%
3	18.1%	5.0%	0%	76.9%
4	6.2%	0.7%	0%	93.1%
5	3.3%	0.7%	0%	96.1%
6	0.8%	0.7%	0%	98.5%
7	0.4%	0.5%	0%	99.1%
8	1.0%	0.4%	0%	98.5%
9	1.0%	0.5%	0%	98.6%
10	13.2%	2.1%	0%	84.6%
11	11.3%	0.8%	0%	87.9%
12	26.3%	5.9%	0%	67.8%
13	28.3%	9.2%	0%	62.6%
14	8.6%	1.1%	0%	90.2%
15	6.4%	0.7%	0%	93.0%
16	2.5%	0.3%	0%	97.2%
17	1.2%	0.2%	0%	98.6%
18	1.4%	0.4%	0%	98.3%
19	1.2%	0.4%	0%	98.4%
20	3.4%	0.4%	0%	96.3%
21	10.9%	1.1%	0%	88.0%
22	31.7%	3.5%	0%	64.8%
23	35.3%	6.2%	0%	58.5%
24	20.0%	6.3%	0%	73.8%
25	21.7%	12.0%	0%	66.3%
26	5.5%	0.7%	0%	93.8%
27	1.1%	0.2%	0%	98.6%
28	1.2%	0.3%	0%	98.5%
29	1.4%	0.7%	0%	97.9%
30	1.3%	0.3%	0%	98.4%
31	0.6%	0.5%	0%	98.9%
32	19.0%	1.9%	0%	79.1%
33	38.0%	7.1%	0%	54.9%
34	16.6%	3.1%	0%	80.3%
35	14.0%	1.7%	0%	84.3%
36	5.9%	0.5%	0%	93.6%
37	0.9%	0.5%	0%	98.6%
38	0.5%	0.4%	0%	99.1%
39	0.9%	0.5%	0%	98.6%
40	2.1%	0.3%	0%	97.6%

41	1.5%	0.7%	0%	97.8%
42	2.0%	0.2%	0%	97.8%
43	24.9%	2.9%	0%	72.3%
44	26.5%	2.8%	0%	70.7%
45	1.8%	0.4%	0%	97.8%
46	0.5%	0.5%	0%	99.0%
47	1.5%	0.4%	0%	98.2%
48	5.3%	0.4%	0%	94.4%
49	4.7%	0.4%	0%	94.9%
50	2.8%	0.2%	0%	97.0%
51	1.1%	0.4%	0%	98.5%
52	3.1%	0.3%	0%	96.6%
53	12.8%	0.5%	0%	86.7%
54	28.8%	4.3%	0%	66.9%
55	10.4%	0.6%	0%	88.9%
56	2.3%	0.3%	0%	97.4%
57	2.7%	1.1%	0%	96.3%
58	2.2%	0.5%	0%	97.3%
59	3.6%	0.3%	0%	96.1%
60	1.3%	0.4%	0%	98.3%
61	0.5%	0.9%	0%	98.6%
62	0.6%	0.6%	0%	98.8%
63	0.5%	1.2%	0%	98.2%
64	12.2%	0.8%	0%	87.0%
65	31.8%	5.0%	0%	63.2%
66	11.2%	0.5%	0%	88.3%
67	1.1%	0.2%	0%	98.7%
68	1.4%	0.5%	0%	98.1%
69	0.7%	0.4%	0%	98.9%
70	1.4%	0.2%	0%	98.4%
71	0.6%	0.2%	0%	99.2%
72	3.2%	0.1%	0%	96.7%
73	1.5%	0.3%	0%	98.2%
74	0.7%	0.5%	0%	98.7%
75	17.9%	1.4%	0%	80.7%
76	22.1%	5.8%	0%	72.1%
77	23.0%	2.9%	0%	74.1%
78	3.6%	0.4%	0%	96.0%
79	7.5%	1.2%	0%	91.4%
80	1.4%	0.4%	0%	98.3%
81	3.4%	0.3%	0%	96.3%
82	4.0%	0.4%	0%	95.6%
83	1.0%	0.8%	0%	98.2%
84	0.9%	0.5%	0%	98.6%
85	16.5%	2.5%	0%	80.9%
86	33.6%	5.7%	0%	60.7%
87	21.3%	7.1%	0%	71.6%
88	5.0%	0.3%	0%	94.8%

89	2.8%	0.3%	0%	96.9%
90	1.1%	0.4%	0%	98.5%
91	0.6%	0.8%	0%	98.6%
92	0.4%	0.5%	0%	99.1%
93	2.1%	1.0%	0%	96.9%
94	3.1%	0.4%	0%	96.5%
95	11.3%	1.4%	0%	87.3%
96	23.8%	12.6%	0%	63.6%
97	35.4%	8.8%	0%	55.9%
98	11.9%	1.0%	0%	87.2%
99	0.8%	0.4%	0%	98.8%

Table 4. Area statistics for biofilm images after 6 hours.

Image number	Cells	Matrix	Channels	Cells-free zone
0	1.8%	0.3%	0%	97.9%
1	0.9%	0.8%	0.0%	98.3%
2	0.9%	0.8%	0%	98.4%
3	0.8%	0.8%	0%	98.4%
4	10.9%	0.7%	0%	88.3%
5	10.7%	5.4%	0%	83.9%
6	13.0%	2.2%	0%	84.8%
7	7.4%	1.4%	0%	91.2%
8	2.5%	0.5%	0%	97.0%
9	6.6%	0.3%	0%	93.1%
10	2.1%	0.5%	0%	97.4%
11	2.3%	0.4%	0%	97.3%
12	0.7%	0.7%	0%	98.7%
13	1.1%	0.4%	0%	98.5%
14	18.8%	2.5%	0%	78.7%
15	19.1%	15.2%	0%	65.7%
16	10.5%	2.2%	0%	87.3%
17	14.0%	1.8%	0%	84.2%
18	3.5%	0.6%	0%	95.9%
19	2.3%	0.8%	0%	96.9%
20	1.2%	0.1%	0%	98.7%
21	1.2%	0.1%	0%	98.7%
22	2.0%	0.7%	0%	97.3%
23	3.3%	0.4%	0%	96.2%
24	6.2%	0.7%	0%	93.2%
25	24.1%	3.3%	0%	72.6%
26	3.7%	0.6%	0%	95.7%
27	1.5%	1.4%	0%	97.1%
28	14.9%	3.6%	0%	81.5%
29	14.0%	0.4%	0%	85.6%
30	3.3%	0.1%	0%	96.6%
31	14.0%	0.8%	0%	85.2%

32	0.6%	0.6%	0%	98.7%
33	2.3%	0.3%	0%	97.3%
34	2.5%	0.3%	0%	97.2%
35	1.9%	0.3%	0%	97.8%
36	2.1%	0.3%	0%	97.6%
37	2.5%	0.4%	0%	97.0%
38	0.7%	0.4%	0%	98.9%
39	6.9%	0.4%	0%	92.7%
40	1.8%	0.2%	0%	98.0%
41	6.0%	0.3%	0%	93.7%
42	1.0%	0.5%	0%	98.5%
43	0.5%	1.0%	0%	98.5%
44	0.8%	1.0%	0%	98.1%
45	0.5%	0.5%	0%	99.0%
46	3.6%	2.1%	0%	94.3%
47	5.2%	1.5%	0%	93.2%
48	1.8%	0.5%	0%	97.7%
49	1.6%	0.5%	0%	97.9%
50	0.8%	0.5%	0%	98.8%
51	1.2%	0.4%	0%	98.5%
52	1.9%	0.5%	0%	97.6%
53	0.8%	1.2%	0%	98.0%
54	3.1%	0.3%	0%	96.6%
55	4.9%	0.6%	0%	94.5%
56	0.6%	0.4%	0%	99.0%
57	2.2%	0.6%	0%	97.2%
58	2.5%	0.4%	0%	97.2%
59	1.5%	0.3%	0%	98.2%
60	1.7%	0.3%	0%	98.0%
61	3.3%	0.4%	0%	96.4%
62	1.6%	0.6%	0%	97.9%
63	1.0%	1.2%	0%	97.8%
64	0.9%	0.4%	0%	98.7%
65	7.7%	0.2%	0%	92.1%
66	6.5%	0.2%	0%	93.2%
67	3.4%	0.6%	0%	96.0%
68	2.9%	0.7%	0%	96.4%
69	4.4%	0.5%	0%	95.1%
70	0.7%	0.6%	0%	98.6%
71	0.9%	0.7%	0%	98.4%
72	1.3%	0.3%	0%	98.4%
73	4.0%	0.5%	0%	95.5%
74	4.8%	0.4%	0%	94.8%
75	4.2%	0.6%	0%	95.2%
76	3.5%	0.3%	0%	96.2%
77	5.0%	0.2%	0%	94.8%
78	3.4%	0.3%	0%	96.3%
79	2.6%	2.0%	0%	95.4%

80	0.8%	0.8%	0%	98.4%
81	1.2%	0.5%	0%	98.3%
82	0.7%	0.2%	0%	99.1%
83	7.7%	0.6%	0%	91.8%
84	6.6%	0.4%	0%	93.0%
85	1.6%	0.3%	0%	98.0%
86	6.9%	0.3%	0%	92.8%
87	7.9%	0.3%	0%	91.8%
88	7.2%	0.3%	0%	92.5%
89	1.2%	0.6%	0%	98.2%
90	1.0%	1.5%	0%	97.5%
91	0.7%	1.4%	0%	97.9%
92	2.4%	0.2%	0%	97.4%
93	18.8%	3.2%	0%	78.0%
94	2.6%	0.4%	0%	97.0%
95	3.9%	0.5%	0%	95.6%
96	22.1%	3.0%	0%	75.0%
97	22.9%	2.3%	0%	74.8%
98	23.2%	3.1%	0%	73.7%
99	11.7%	0.6%	0%	87.8%

Table 5. Area statistics for biofilm images after 9 hours.

Image number	Cells	Matrix	Channels	Cells-free zone
0	2.3%	0.3%	0%	97.4%
1	33.1%	7.4%	0%	59.5%
2	39.3%	11.6%	0%	49.1%
3	40.6%	12.1%	0%	47.3%
4	57.3%	17.6%	0.1%	25.0%
5	59.4%	28.7%	0%	11.8%
6	56.4%	30.8%	0%	12.8%
7	49.8%	11.9%	0.1%	38.2%
8	35.6%	3.5%	0%	60.9%
9	41.1%	5.7%	0%	53.2%
10	14.2%	0.7%	0%	85.1%
11	36.6%	3.7%	0%	59.7%
12	39.5%	8.1%	0%	52.5%
13	41.0%	7.8%	0%	51.3%
14	54.5%	19.4%	0.1%	26.0%
15	58.6%	28.6%	0.1%	12.7%
16	56.3%	26.2%	0%	17.5%
17	34.1%	3.9%	0%	62.0%
18	10.2%	0.3%	0%	89.5%
19	35.6%	3.9%	0%	60.5%
20	35.5%	3.4%	0%	61.0%
21	45.4%	5.9%	0%	48.7%
22	36.3%	4.0%	0%	59.8%

23	40.5%	5.5%	0%	54.0%
24	57.1%	24.1%	0.1%	18.7%
25	57.5%	31.3%	0.4%	10.8%
26	47.3%	10.7%	0%	42.0%
27	3.5%	0.3%	0%	96.2%
28	0.7%	0.9%	0%	98.4%
29	13.8%	0.5%	0%	85.7%
30	36.6%	8.1%	0%	55.3%
31	40.4%	5.7%	0%	54.0%
32	35.1%	3.5%	0%	61.4%
33	46.7%	9.3%	0%	44.0%
34	60.6%	28.1%	0%	11.3%
35	57.9%	32.0%	0%	10.1%
36	39.2%	4.7%	0%	56.1%
37	13.5%	0.9%	0%	85.6%
38	10.1%	0.5%	0%	89.3%
39	1.2%	0.4%	0%	98.5%
40	39.6%	8.7%	0%	51.6%
41	36.3%	5.7%	0%	58.0%
42	40.2%	6.2%	0%	53.5%
43	51.7%	15.0%	0.3%	32.9%
44	59.9%	28.1%	0.2%	11.9%
45	56.3%	26.4%	0%	17.3%
46	32.0%	4.0%	0%	64.0%
47	38.3%	10.7%	0%	51.0%
48	20.9%	1.4%	0%	77.7%
49	15.7%	0.6%	0%	83.7%
50	45.2%	6.5%	0%	48.3%
51	41.2%	7.8%	0%	51.0%
52	38.4%	10.0%	0%	51.6%
53	56.2%	16.3%	0.3%	27.1%
54	60.6%	29.6%	0.1%	9.8%
55	56.8%	17.6%	0.1%	25.5%
56	44.7%	7.8%	0%	47.5%
57	38.3%	10.7%	0%	51.0%
58	41.5%	13.9%	0%	44.6%
59	44.3%	7.0%	0%	48.7%
60	39.6%	18.2%	0%	42.2%
61	28.1%	2.1%	0%	69.9%
62	1.0%	1.2%	0%	97.7%
63	52.4%	18.2%	0.5%	28.9%
64	55.7%	31.6%	0%	12.7%
65	46.5%	7.7%	0.1%	45.8%
66	37.9%	3.9%	0%	58.2%
67	44.0%	6.2%	0%	49.8%
68	41.4%	7.6%	0%	51.0%
69	40.2%	5.5%	0%	54.3%
70	1.7%	0.8%	0%	97.5%

71	2.4%	0.6%	0%	97.0%
72	0.5%	0.7%	0%	98.8%
73	42.9%	6.8%	0%	50.3%
74	60.4%	26.3%	0.1%	13.3%
75	30.0%	2.3%	0%	67.7%
76	6.4%	0.3%	0%	93.3%
77	36.3%	3.7%	0%	60.1%
78	44.8%	11.6%	0%	43.6%
79	42.3%	5.9%	0%	51.7%
80	0.7%	0.9%	0%	98.4%
81	0.3%	1.0%	0%	98.7%
82	5.7%	0.3%	0%	94.0%
83	33.7%	10.8%	0%	55.5%
84	56.3%	16.6%	0.2%	26.9%
85	40.5%	6.2%	0.4%	52.8%
86	0.8%	0.7%	0%	98.4%
87	2.1%	0.3%	0%	97.6%
88	37.3%	4.7%	0%	58.0%
89	51.5%	14.9%	0%	33.6%
90	13.0%	0.6%	0%	86.4%
91	0.5%	0.3%	0%	99.2%
92	23.6%	2.2%	0%	74.2%
93	4.8%	0.3%	0%	94.9%
94	5.8%	0.3%	0%	93.9%
95	53.2%	10.5%	0.5%	35.8%
96	17.1%	1.3%	0%	81.5%
97	8.9%	0.5%	0%	90.6%
98	50.7%	12.3%	0%	37.0%
99	57.8%	29.0%	0.1%	13.1%

Table 6. Area statistics for biofilm images after 24 hours.

Image number	Cells	Matrix	Channels	Cells-free zone
0	6.4%	0.4%	0%	93.2%
1	56.9%	25.3%	0.2%	17.6%
2	59.6%	29.2%	0.3%	10.9%
3	1.2%	0.3%	0%	98.5%
4	33.8%	2.2%	0%	64.0%
5	49.6%	10.3%	0%	40.1%
6	54.0%	17.0%	0.3%	28.8%
7	39.0%	4.3%	0%	56.7%
8	50.6%	10.8%	0%	38.6%
9	53.0%	19.5%	0%	27.5%
10	45.1%	7.1%	0.1%	47.7%
11	55.0%	17.4%	0%	27.6%
12	38.2%	8.5%	0%	53.3%
13	50.6%	11.8%	0%	37.5%

14	49.1%	11.9%	0%	39.0%
15	46.5%	9.5%	0%	44.0%
16	52.1%	14.0%	0%	34.0%
17	53.3%	16.6%	0.2%	29.9%
18	51.4%	11.8%	0%	36.9%
19	49.0%	10.1%	0%	40.9%
20	11.1%	0.3%	0%	88.6%
21	7.0%	0.4%	0%	92.6%
22	13.1%	0.4%	0%	86.5%
23	47.3%	9.0%	0%	43.7%
24	49.8%	10.3%	0%	40.0%
25	54.6%	17.1%	0.2%	28.1%
26	50.0%	14.4%	0%	35.6%
27	50.4%	9.7%	0%	39.9%
28	47.0%	11.3%	0%	41.6%
29	36.0%	3.5%	0%	60.5%
30	11.6%	0.1%	0%	88.2%
31	0.4%	1.0%	0%	98.6%
32	50.1%	9.4%	0.1%	40.5%
33	4.0%	0.2%	0%	95.9%
34	48.0%	9.7%	0%	42.3%
35	48.8%	9.6%	0%	41.6%
36	48.0%	13.5%	0%	38.5%
37	49.9%	10.2%	0%	39.9%
38	53.9%	16.4%	0%	29.7%
39	2.7%	0.6%	0%	96.7%
40	1.0%	0.6%	0%	98.5%
41	8.6%	0.3%	0%	91.2%
42	25.6%	2.1%	0%	72.3%
43	20.8%	0.9%	0%	78.3%
44	50.6%	11.8%	0%	37.5%
45	50.2%	14.0%	0%	35.7%
46	47.9%	10.4%	0%	41.7%
47	49.3%	12.3%	0%	38.4%
48	31.8%	6.0%	0%	62.2%
49	1.0%	0.7%	0%	98.3%
50	0.5%	0.8%	0%	98.7%
51	24.7%	1.3%	0%	74.1%
52	23.7%	2.2%	0%	74.2%
53	45.8%	7.8%	0%	46.4%
54	49.8%	9.9%	0%	40.4%
55	52.1%	14.0%	0%	33.9%
56	49.2%	11.5%	0%	39.3%
57	56.7%	20.0%	0%	23.3%
58	47.6%	7.0%	0.2%	45.2%
59	2.9%	0.2%	0%	96.9%
60	0.6%	1.4%	0%	98.1%
61	1.2%	0.9%	0%	97.9%

62	2.2%	0.4%	0%	97.4%
63	49.9%	10.2%	0.1%	39.8%
64	49.5%	10.4%	0%	40.1%
65	50.1%	12.9%	0%	36.9%
66	48.1%	9.6%	0%	42.3%
67	58.6%	26.0%	0%	15.4%
68	55.9%	23.0%	0.1%	21.0%
69	1.8%	0.3%	0%	97.8%
70	1.9%	0.4%	0%	97.7%
71	54.4%	16.1%	0%	29.5%
72	48.9%	11.0%	0.1%	40.0%
73	47.2%	12.3%	0%	40.4%
74	52.6%	15.3%	0%	32.1%
75	49.0%	8.2%	0%	42.7%
76	47.8%	9.1%	0%	43.2%
77	59.7%	23.3%	0.1%	16.9%
78	62.2%	28.6%	0%	9.2%
79	0.6%	0.9%	0%	98.5%
80	36.2%	17.2%	0%	46.7%
81	16.0%	2.4%	0%	81.6%
82	48.9%	9.3%	0%	41.9%
83	54.5%	16.2%	0%	29.4%
84	48.3%	9.6%	0%	42.0%
85	50.6%	11.0%	0%	38.4%
86	49.9%	11.3%	0%	38.8%
87	59.7%	27.9%	0.1%	12.3%
88	57.5%	15.3%	0%	27.1%
89	0.7%	1.6%	0%	97.7%
90	30.2%	4.3%	0%	65.4%
91	1.0%	1.2%	0%	97.9%
92	47.9%	8.1%	0%	44.0%
93	50.0%	11.2%	0%	38.9%
94	47.2%	9.6%	0%	43.2%
95	50.6%	9.3%	0%	40.1%
96	49.0%	10.9%	0%	40.0%
97	47.7%	8.5%	0%	43.8%
98	59.2%	24.9%	0%	16.0%
99	49.3%	11.1%	0%	39.5%

Table 7. Area statistics for biofilm images after 48 hours.

Image number	Cells	Matrix	Channels	Cells-free zone
0	42.1%	21.4%	0%	36.5%
1	53.8%	30.3%	0%	15.9%
2	57.3%	24.1%	0%	18.6%
3	59.4%	31.1%	0.1%	9.5%
4	51.8%	29.3%	0%	18.8%

5	56.6%	26.5%	0%	17.0%
6	43.4%	11.8%	0%	44.8%
7	58.1%	33.6%	0%	8.2%
8	55.7%	34.8%	0%	9.5%
9	55.7%	27.6%	0%	16.7%
10	47.4%	26.5%	0%	26.1%
11	55.5%	29.2%	0%	15.3%
12	55.6%	27.1%	0%	17.3%
13	50.9%	26.1%	0%	22.9%
14	58.3%	34.4%	0.4%	6.8%
15	62.1%	28.0%	0.2%	9.8%
16	56.2%	33.4%	0%	10.4%
17	54.4%	25.1%	0%	20.5%
18	50.4%	25.0%	0%	24.6%
19	55.9%	26.0%	0.4%	17.8%
20	55.2%	31.3%	0%	13.4%
21	56.2%	31.3%	0%	12.5%
22	56.4%	29.8%	0%	13.8%
23	50.3%	18.7%	0%	30.9%
24	53.2%	35.4%	0%	11.4%
25	56.1%	31.1%	0%	12.7%
26	54.3%	37.1%	0.1%	8.4%
27	46.2%	13.0%	0%	40.8%
28	56.8%	28.5%	0%	14.7%
29	56.4%	31.1%	0.1%	12.4%
30	55.0%	29.2%	0%	15.8%
31	56.7%	31.4%	0.2%	11.7%
32	57.8%	27.0%	0%	15.2%
33	55.8%	25.8%	0%	18.4%
34	45.2%	19.6%	0%	35.2%
35	56.5%	33.6%	0.2%	9.8%
36	59.1%	33.0%	0.1%	7.8%
37	56.8%	33.8%	0.2%	9.1%
38	56.1%	31.3%	0%	12.6%
39	52.8%	28.5%	0%	18.6%
40	56.6%	30.3%	0%	13.1%
41	55.9%	32.5%	0%	11.5%
42	57.3%	26.0%	0%	16.7%
43	52.7%	19.7%	0%	27.6%
44	52.7%	27.7%	0%	19.6%
45	6.9%	0.2%	0%	92.8%
46	14.1%	2.0%	0%	83.9%
47	57.4%	32.2%	0%	10.4%
48	57.7%	24.4%	0%	17.9%
49	52.0%	23.0%	0%	24.9%
50	59.3%	30.2%	0.1%	10.5%
51	57.7%	31.0%	0.2%	11.1%
52	50.4%	20.1%	0.1%	29.4%

53	49.9%	13.0%	0%	37.1%
54	48.3%	23.5%	0%	28.2%
55	56.4%	36.3%	0.2%	7.2%
56	53.5%	35.8%	0%	10.7%
57	56.4%	27.6%	0%	16.0%
58	56.7%	31.5%	0.2%	11.7%
59	56.6%	29.9%	0%	13.5%
60	59.6%	28.5%	0%	11.9%
61	59.0%	29.5%	0%	11.5%
62	52.8%	15.6%	0%	31.5%
63	50.9%	14.5%	0%	34.5%
64	52.8%	19.7%	0%	27.5%
65	60.0%	28.6%	0.4%	11.1%
66	48.1%	17.3%	0%	34.6%
67	52.9%	31.1%	0%	16.1%
68	60.2%	29.7%	0%	10.1%
69	54.5%	25.3%	0%	20.2%
70	55.7%	30.7%	0%	13.6%
71	57.3%	31.0%	0%	11.7%
72	56.6%	30.6%	0%	12.8%
73	50.4%	17.9%	0%	31.7%
74	57.7%	27.2%	0%	15.1%
75	48.1%	25.7%	0%	26.2%
76	35.2%	6.9%	0%	58.0%
77	53.8%	28.7%	0%	17.5%
78	56.4%	27.4%	0%	16.2%
79	50.3%	17.3%	0%	32.4%
80	55.3%	23.3%	0%	21.5%
81	57.1%	24.9%	0.1%	17.9%
82	57.4%	29.2%	0.2%	13.2%
83	52.1%	29.7%	0%	18.3%
84	52.9%	21.1%	0%	26.0%
85	65.4%	18.9%	0.2%	15.4%
86	10.9%	2.5%	0%	86.6%
87	54.4%	27.3%	0%	18.3%
88	53.2%	28.4%	0%	18.4%
89	56.5%	30.1%	0%	13.5%
90	49.7%	17.9%	0%	32.4%
91	56.6%	32.3%	0%	11.1%
92	56.1%	30.7%	0%	13.2%
93	58.8%	24.7%	0%	16.5%
94	56.1%	26.8%	0%	17.1%
95	55.8%	36.1%	0%	8.1%
96	58.9%	33.0%	0.1%	8.0%
97	55.8%	25.6%	0%	18.6%
98	56.7%	29.5%	0%	13.8%
99	57.0%	28.3%	0%	14.7%

Table 8. Area statistics for biofilm images after 72 hours.

Image number	Cells	Matrix	Channels	Cells-free zone
0	36.9%	18.8%	0%	44.3%
1	7.6%	0.7%	0%	91.6%
2	8.3%	2.0%	0%	89.7%
3	51.7%	27.1%	0%	21.2%
4	27.0%	10.8%	0%	62.2%
5	25.3%	8.9%	0%	65.8%
6	35.0%	21.8%	0%	43.2%
7	8.9%	1.4%	0%	89.6%
8	28.9%	16.6%	0%	54.5%
9	39.2%	19.9%	0%	40.9%
10	23.3%	19.4%	0%	57.2%
11	13.3%	2.2%	0%	84.6%
12	25.5%	13.4%	0%	61.1%
13	35.8%	13.7%	0%	50.5%
14	52.8%	31.1%	0%	16.1%
15	13.4%	4.3%	0%	82.3%
16	43.3%	24.4%	0%	32.3%
17	54.2%	24.3%	0.1%	21.4%
18	33.5%	21.0%	0%	45.5%
19	31.2%	20.0%	0%	48.8%
20	28.5%	15.7%	0%	55.8%
21	18.6%	4.4%	0%	77.0%
22	32.4%	11.6%	0%	56.0%
23	16.2%	10.8%	0%	73.0%
24	44.6%	25.9%	0%	29.4%
25	47.6%	23.1%	0%	29.4%
26	7.1%	1.8%	0%	91.1%
27	43.8%	25.6%	0%	30.7%
28	52.8%	25.5%	0%	21.7%
29	49.1%	22.1%	0%	28.8%
30	26.9%	15.7%	0%	57.4%
31	24.4%	7.2%	0%	68.5%
32	12.8%	3.0%	0%	84.1%
33	27.5%	28.9%	0%	43.5%
34	31.2%	17.8%	0%	51.0%
35	36.7%	24.1%	0%	39.2%
36	55.0%	29.4%	0%	15.6%
37	13.3%	11.5%	0%	75.2%
38	45.7%	26.7%	0%	27.6%
39	54.6%	29.9%	0%	15.5%
40	20.9%	6.2%	0%	72.9%
41	28.6%	18.8%	0%	52.6%
42	19.3%	10.7%	0%	70.1%
43	15.4%	18.6%	0%	66.0%

44	24.9%	13.4%	0%	61.7%
45	26.8%	16.6%	0%	56.6%
46	35.9%	14.7%	0%	49.5%
47	52.9%	28.4%	0.2%	18.6%
48	31.3%	17.2%	0%	51.4%
49	49.5%	22.2%	0%	28.3%
50	38.2%	24.9%	0%	36.9%
51	29.8%	17.1%	0%	53.1%
52	28.8%	22.0%	0%	49.2%
53	35.2%	23.4%	0%	41.3%
54	40.1%	30.6%	0%	29.3%
55	27.9%	17.1%	0%	55.1%
56	14.4%	4.8%	0%	80.8%
57	29.5%	15.9%	0%	54.6%
58	51.2%	29.8%	0%	19.0%
59	42.2%	16.9%	0%	40.9%
60	57.3%	33.8%	0%	8.8%
61	46.6%	27.5%	0%	25.9%
62	30.4%	26.4%	0%	43.2%
63	31.5%	21.6%	0%	46.8%
64	44.7%	28.3%	0%	27.1%
65	28.9%	31.2%	0%	39.8%
66	18.0%	6.6%	0%	75.4%
67	20.4%	9.0%	0%	70.6%
68	35.1%	19.8%	0%	45.0%
69	50.5%	27.3%	0%	22.1%
70	55.1%	34.6%	0%	10.2%
71	53.4%	39.5%	0%	7.1%
72	52.8%	36.4%	0%	10.7%
73	39.1%	24.6%	0%	36.3%
74	33.8%	25.1%	0%	41.1%
75	51.1%	25.2%	0%	23.8%
76	33.7%	14.9%	0%	51.3%
77	48.1%	14.4%	0%	37.5%
78	17.4%	9.5%	0%	73.1%
79	39.3%	26.9%	0%	33.8%
80	0.3%	0.6%	0%	99.1%
81	47.7%	21.3%	0.2%	30.8%
82	55.6%	37.4%	0.1%	6.9%
83	54.0%	32.4%	0%	13.6%
84	52.4%	26.4%	0.1%	21.1%
85	44.8%	29.9%	0%	25.3%
86	43.8%	15.9%	0%	40.3%
87	22.0%	23.7%	0%	54.3%
88	45.3%	29.3%	0%	25.4%
89	35.8%	9.9%	0%	54.3%
90	0.4%	0.5%	0%	99.1%
91	0.3%	0.5%	0%	99.2%

92	20.5%	2.9%	0%	76.6%
93	55.3%	36.3%	0.1%	8.3%
94	55.9%	36.6%	0%	7.5%
95	52.8%	36.4%	0%	10.8%
96	50.8%	34.6%	0%	14.6%
97	37.1%	20.0%	0%	42.9%
98	46.9%	16.1%	0%	37.0%
99	52.2%	26.0%	0.0%	21.7%

Table 9. Area statistics for biofilm images after 96 hours.

Image number	Cells	Matrix	Channels	Cells-free zone
0	6.4%	0.6%	0%	93.0%
1	12.9%	3.3%	0%	83.8%
2	7.4%	2.0%	0%	90.6%
3	23.6%	17.8%	0%	58.6%
4	56.8%	31.3%	0.1%	11.9%
5	56.3%	36.1%	0.1%	7.5%
6	57.1%	33.6%	0.1%	9.2%
7	53.6%	32.9%	0.1%	13.5%
8	55.5%	34.1%	0%	10.4%
9	56.8%	32.6%	0.1%	10.4%
10	6.3%	1.8%	0%	91.9%
11	5.7%	1.4%	0%	92.9%
12	10.5%	6.2%	0%	83.3%
13	26.8%	11.4%	0%	61.9%
14	54.7%	37.1%	0.1%	8.1%
15	54.8%	31.7%	0%	13.5%
16	55.4%	37.5%	0.2%	6.9%
17	55.5%	36.2%	0.1%	8.2%
18	54.2%	34.2%	0%	11.5%
19	57.7%	31.8%	0%	10.5%
20	7.1%	1.8%	0%	91.1%
21	8.3%	4.3%	0%	87.5%
22	23.6%	12.8%	0%	63.7%
23	36.9%	30.3%	0%	32.8%
24	51.8%	34.5%	0%	13.7%
25	56.0%	34.7%	0%	9.3%
26	52.3%	37.0%	0%	10.7%
27	52.5%	36.0%	0%	11.5%
28	55.1%	35.3%	0.3%	9.3%
29	57.4%	33.3%	0.4%	8.9%
30	6.8%	0.7%	0%	92.5%
31	9.6%	2.5%	0%	88.0%
32	24.3%	13.3%	0%	62.4%
33	47.0%	29.2%	0%	23.8%
34	54.2%	36.0%	0.1%	9.7%

35	53.2%	34.3%	0%	12.5%
36	56.0%	37.0%	0%	7.0%
37	52.6%	39.3%	0%	8.1%
38	56.6%	36.6%	0.1%	6.8%
39	58.7%	33.6%	0.1%	7.6%
40	30.2%	22.0%	0%	47.8%
41	31.2%	23.9%	0%	44.9%
42	19.2%	12.8%	0%	68.0%
43	43.7%	24.0%	0%	32.2%
44	54.3%	31.0%	0%	14.7%
45	55.3%	35.8%	0%	9.0%
46	56.5%	36.9%	0.1%	6.5%
47	51.5%	37.6%	0.1%	10.8%
48	53.4%	36.7%	0%	9.9%
49	52.6%	36.3%	0%	11.1%
50	45.9%	12.1%	0%	42.1%
51	27.8%	9.0%	0%	63.1%
52	32.2%	27.3%	0%	40.5%
53	39.1%	31.1%	0%	29.8%
54	51.7%	28.0%	0%	20.3%
55	54.4%	36.1%	0%	9.5%
56	52.8%	36.7%	0.1%	10.4%
57	46.4%	29.9%	0%	23.7%
58	49.2%	32.9%	0%	17.8%
59	49.5%	35.0%	0%	15.5%
60	55.5%	36.0%	0.2%	8.2%
61	32.7%	20.2%	0%	47.1%
62	40.8%	26.9%	0%	32.3%
63	36.2%	31.2%	0%	32.5%
64	49.5%	31.8%	0.1%	18.6%
65	54.1%	25.4%	0%	20.5%
66	48.2%	23.9%	0%	27.9%
67	47.4%	24.8%	0%	27.8%
68	50.4%	27.7%	0%	21.9%
69	47.8%	30.9%	0%	21.3%
70	56.0%	34.8%	0.1%	9.1%
71	34.0%	17.3%	0%	48.6%
72	20.5%	11.8%	0%	67.7%
73	45.6%	33.0%	0%	21.4%
74	42.5%	34.8%	0%	22.6%
75	37.8%	28.0%	0%	34.2%
76	42.0%	19.2%	0%	38.8%
77	40.5%	20.4%	0%	39.1%
78	47.6%	32.6%	0%	19.8%
79	51.1%	25.2%	0%	23.7%
80	53.4%	37.2%	0.1%	9.3%
81	31.6%	24.7%	0%	43.7%
82	33.9%	26.4%	0%	39.7%

83	45.1%	30.3%	0%	24.6%
84	49.0%	29.7%	0%	21.3%
85	37.1%	21.5%	0%	41.3%
86	42.1%	26.0%	0%	31.8%
87	45.9%	25.8%	0%	28.3%
88	52.0%	27.6%	0%	20.4%
89	50.2%	37.1%	0%	12.7%
90	54.6%	36.4%	0%	8.9%
91	39.2%	20.8%	0%	40.0%
92	35.4%	28.6%	0%	36.0%
93	39.7%	27.3%	0%	33.0%
94	45.3%	24.8%	0%	29.9%
95	35.8%	20.6%	0%	43.6%
96	45.5%	25.5%	0%	29.0%
97	49.6%	31.0%	0%	19.4%
98	53.5%	34.3%	0%	12.2%
99	49.0%	32.3%	0%	18.7%

3.5 Neural network-based predictions of large amounts of SEM images for the study of biocide effects

Here, we report statistics of segmentation class areas and cell counts on each image, which were used in the comparison of different antibiotic impacts.

Table 10. Statistics of Benzalkonium chloride impact.

Image number	Cells	Matrix	Cells-free zone	Number of cells
0	39.8%	9.5%	50.7%	137
1	41.2%	11.2%	47.6%	125
2	37.0%	11.1%	51.9%	112
3	28.7%	4.1%	67.2%	79
4	41.8%	5.8%	52.4%	129
5	37.1%	8.5%	54.4%	107
6	23.2%	3.6%	73.2%	71
7	41.3%	7.9%	50.8%	135
8	40.1%	17.4%	42.4%	133
9	38.7%	12.2%	49.0%	115
10	37.9%	7.2%	54.9%	111
11	39.4%	7.0%	53.6%	114
12	36.1%	10.0%	54.0%	103
13	22.3%	11.3%	66.4%	66
14	42.9%	7.0%	50.1%	131
15	29.2%	49.6%	21.2%	63
16	27.1%	4.9%	68.0%	79
17	40.6%	7.4%	52.0%	131
18	38.8%	10.6%	50.6%	123
19	38.0%	6.9%	55.1%	116
20	7.1%	2.1%	90.8%	21
21	40.7%	9.5%	49.8%	120

22	32.0%	7.7%	60.3%	90
23	30.5%	17.5%	52.0%	94
24	39.5%	11.1%	49.3%	125
25	27.5%	24.5%	48.0%	81
26	25.2%	40.3%	34.4%	75
27	38.7%	16.4%	44.8%	115
28	30.1%	34.2%	35.7%	96
29	39.1%	9.0%	51.9%	116
30	0.3%	0.4%	99.3%	4
31	36.4%	19.4%	44.2%	109
32	38.3%	10.1%	51.6%	113
33	34.3%	14.1%	51.6%	102
34	39.6%	13.8%	46.6%	127
35	6.9%	3.3%	89.8%	22
36	13.8%	20.1%	66.0%	49
37	38.5%	7.1%	54.4%	122
38	40.0%	7.4%	52.7%	133
39	41.6%	11.5%	46.8%	126
40	0.4%	1.1%	98.5%	4
41	22.9%	37.7%	39.3%	73
42	39.3%	8.3%	52.5%	123
43	37.8%	14.3%	47.9%	113
44	22.5%	44.5%	33.0%	65
45	38.3%	19.7%	42.1%	114
46	38.1%	9.9%	52.0%	109
47	41.2%	6.0%	52.8%	131
48	42.1%	10.3%	47.7%	141
49	39.4%	9.7%	50.9%	125
50	0.7%	0.5%	98.8%	5
51	19.7%	15.3%	65.0%	65
52	33.9%	9.6%	56.5%	99
53	33.9%	13.6%	52.5%	107
54	40.9%	14.0%	45.0%	134
55	41.0%	9.8%	49.2%	133
56	41.4%	9.7%	48.9%	130
57	41.8%	7.6%	50.7%	128
58	11.7%	1.5%	86.8%	38
59	38.2%	7.4%	54.5%	114
60	0.5%	0.5%	99.0%	5
61	17.2%	1.5%	81.2%	53
62	34.3%	6.0%	59.7%	101
63	36.8%	4.4%	58.8%	109
64	42.5%	8.7%	48.8%	129
65	38.7%	11.3%	50.0%	123
66	38.7%	11.5%	49.9%	118
67	42.6%	12.5%	44.9%	132
68	40.4%	6.9%	52.7%	125
69	37.6%	9.0%	53.4%	109

70	0.5%	0.3%	99.1%	4
71	18.1%	2.0%	79.9%	55
72	34.7%	6.5%	58.8%	104
73	43.5%	8.8%	47.7%	133
74	42.3%	10.2%	47.5%	137
75	39.5%	9.0%	51.5%	127
76	12.6%	13.6%	73.8%	44
77	40.6%	8.0%	51.4%	129
78	38.7%	7.8%	53.5%	117
79	39.8%	9.3%	50.9%	121
80	0.5%	0.8%	98.6%	6
81	24.0%	2.4%	73.6%	82
82	36.8%	9.8%	53.4%	106
83	37.7%	9.7%	52.5%	126
84	21.5%	35.5%	43.0%	56
85	39.3%	9.5%	51.2%	121
86	42.3%	8.1%	49.6%	140
87	36.9%	6.2%	56.9%	115
88	41.4%	11.3%	47.4%	128
89	38.2%	45.8%	16.0%	102
90	0.7%	0.6%	98.8%	4
91	24.2%	2.2%	73.6%	77
92	29.1%	50.3%	20.6%	83
93	40.2%	7.2%	52.6%	130
94	39.3%	9.1%	51.6%	122
95	39.2%	8.0%	52.8%	124
96	39.4%	5.9%	54.7%	122
97	35.5%	28.4%	36.0%	112
98	26.9%	44.0%	29.0%	88
99	24.9%	40.9%	34.2%	76

Table 11. Statistics of Gentamycin impact.

Image number	Cells	Matrix	Cells-free zone	Number of cells
0	9.7%	0.6%	89.7%	32
1	32.1%	7.1%	60.9%	107
2	17.1%	6.9%	76.0%	63
3	13.8%	3.6%	82.5%	42
4	27.8%	10.7%	61.5%	85
5	21.0%	1.9%	77.1%	58
6	35.9%	4.9%	59.2%	125
7	31.9%	2.2%	65.9%	111
8	29.0%	2.1%	68.9%	93
9	36.1%	5.2%	58.7%	121
10	25.3%	3.4%	71.3%	81
11	31.5%	13.9%	54.6%	109
12	28.8%	3.4%	67.8%	87

13	21.6%	3.6%	74.9%	75
14	29.4%	4.9%	65.7%	97
15	29.6%	3.5%	66.9%	104
16	35.9%	5.6%	58.4%	131
17	31.7%	1.9%	66.4%	97
18	21.4%	1.9%	76.7%	70
19	27.4%	7.0%	65.6%	89
20	34.6%	8.3%	57.1%	107
21	35.4%	6.0%	58.5%	117
22	9.9%	0.8%	89.3%	16
23	20.1%	6.4%	73.6%	66
24	26.7%	3.3%	70.0%	88
25	29.3%	8.3%	62.4%	98
26	30.5%	7.8%	61.7%	99
27	27.3%	2.2%	70.4%	84
28	22.9%	3.9%	73.2%	70
29	27.6%	26.6%	45.7%	91
30	32.9%	8.7%	58.4%	109
31	34.4%	16.9%	48.6%	119
32	19.8%	2.9%	77.3%	57
33	17.2%	3.6%	79.1%	57
34	27.1%	3.8%	69.1%	91
35	14.0%	0.5%	85.5%	45
36	20.0%	4.7%	75.3%	67
37	28.4%	4.4%	67.2%	91
38	28.0%	3.7%	68.3%	88
39	25.2%	9.4%	65.4%	81
40	34.9%	11.4%	53.7%	102
41	37.0%	6.4%	56.6%	133
42	14.7%	1.8%	83.4%	48
43	7.9%	0.4%	91.7%	28
44	31.2%	3.4%	65.4%	109
45	20.0%	3.0%	77.0%	66
46	25.2%	3.8%	71.0%	77
47	24.9%	2.4%	72.7%	83
48	30.2%	6.7%	63.1%	100
49	31.6%	7.0%	61.4%	95
50	37.6%	6.4%	56.0%	132
51	40.1%	11.4%	48.5%	129
52	22.4%	1.3%	76.4%	77
53	18.6%	3.0%	78.5%	61
54	28.1%	3.5%	68.4%	90
55	29.4%	3.8%	66.8%	92
56	32.3%	6.1%	61.6%	109
57	27.4%	9.8%	62.8%	85
58	26.9%	1.9%	71.2%	87
59	31.1%	12.5%	56.4%	99
60	39.3%	10.3%	50.4%	130

61	39.7%	16.9%	43.4%	134
62	16.0%	1.0%	83.1%	51
63	17.8%	8.2%	74.0%	58
64	31.8%	2.1%	66.2%	103
65	32.2%	10.1%	57.8%	101
66	29.5%	4.5%	66.0%	99
67	25.3%	3.4%	71.3%	84
68	28.7%	4.0%	67.3%	92
69	32.7%	6.3%	61.0%	101
70	44.5%	10.3%	45.2%	148
71	35.6%	6.7%	57.7%	111
72	12.9%	0.3%	86.8%	46
73	25.1%	4.9%	70.1%	85
74	35.5%	2.7%	61.9%	120
75	34.7%	10.6%	54.7%	110
76	26.3%	3.8%	69.9%	81
77	29.6%	4.5%	65.9%	97
78	28.9%	2.7%	68.4%	91
79	24.4%	8.9%	66.7%	71
80	37.7%	5.3%	57.1%	125
81	36.0%	8.5%	55.5%	120
82	23.1%	3.9%	73.1%	77
83	35.5%	6.3%	58.2%	120
84	37.4%	6.0%	56.6%	119
85	35.2%	4.6%	60.2%	112
86	31.4%	4.3%	64.3%	104
87	32.1%	13.3%	54.6%	102
88	31.3%	4.2%	64.5%	101
89	19.7%	3.5%	76.8%	60
90	27.2%	1.3%	71.5%	88
91	23.3%	10.0%	66.7%	76
92	26.8%	5.7%	67.5%	94
93	33.8%	9.4%	56.9%	117
94	36.0%	6.3%	57.7%	124
95	34.8%	10.6%	54.6%	113
96	27.2%	4.0%	68.8%	82
97	30.5%	4.8%	64.6%	97
98	28.6%	3.8%	67.7%	87
99	30.9%	4.0%	65.1%	95

Table 12. Statistics of Chloramphenicol impact.

Image number	Cells	Matrix	Cells-free zone	Number of cells
0	32.1%	3.4%	64.5%	113
1	28.4%	1.0%	70.6%	81
2	20.9%	1.0%	78.1%	66
3	34.7%	6.3%	59.0%	128

4	42.4%	8.3%	49.4%	140
5	57.1%	24.6%	18.3%	206
6	50.0%	16.6%	33.3%	175
7	36.5%	6.3%	57.3%	122
8	41.0%	6.1%	52.9%	144
9	32.5%	2.5%	65.0%	109
10	28.4%	2.1%	69.5%	101
11	37.9%	5.0%	57.1%	130
12	19.7%	1.0%	79.4%	62
13	40.4%	5.4%	54.2%	131
14	41.2%	6.9%	51.9%	133
15	42.8%	7.7%	49.6%	147
16	33.3%	2.6%	64.0%	104
17	37.9%	5.0%	57.1%	127
18	26.2%	1.5%	72.2%	82
19	22.6%	1.5%	75.9%	76
20	34.3%	3.6%	62.1%	121
21	40.9%	6.7%	52.4%	143
22	38.8%	5.5%	55.7%	129
23	38.1%	5.0%	56.9%	130
24	35.0%	3.8%	61.2%	122
25	37.6%	6.2%	56.3%	125
26	32.7%	3.1%	64.1%	105
27	28.5%	2.5%	69.0%	94
28	37.8%	5.0%	57.2%	130
29	28.3%	2.4%	69.3%	90
30	31.0%	2.4%	66.6%	109
31	38.0%	4.5%	57.5%	124
32	44.4%	9.5%	46.0%	149
33	40.7%	6.4%	52.9%	138
34	40.3%	6.2%	53.5%	142
35	32.1%	2.3%	65.6%	103
36	32.3%	2.5%	65.2%	113
37	35.5%	5.0%	59.5%	122
38	24.2%	1.6%	74.1%	78
39	26.9%	2.7%	70.4%	85
40	52.6%	27.4%	20.0%	177
41	47.4%	18.2%	34.4%	164
42	39.9%	17.8%	42.3%	124
43	41.9%	7.2%	50.9%	138
44	43.2%	8.5%	48.3%	144
45	33.3%	2.4%	64.3%	92
46	33.5%	3.4%	63.1%	116
47	36.2%	4.1%	59.7%	129
48	24.7%	0.9%	74.4%	75
49	29.9%	3.1%	67.0%	100
50	28.3%	2.5%	69.2%	92
51	35.4%	5.0%	59.6%	108

52	40.9%	6.7%	52.4%	132
53	31.2%	7.6%	61.3%	101
54	39.2%	5.6%	55.2%	128
55	44.2%	8.1%	47.7%	154
56	38.0%	3.3%	58.7%	123
57	35.9%	4.2%	59.9%	122
58	36.0%	4.4%	59.6%	123
59	38.2%	5.2%	56.6%	138
60	36.7%	5.0%	58.4%	117
61	39.4%	5.4%	55.1%	125
62	27.2%	3.6%	69.2%	84
63	37.5%	5.9%	56.6%	124
64	35.7%	3.9%	60.4%	112
65	37.5%	4.9%	57.7%	120
66	28.8%	2.5%	68.7%	98
67	32.6%	3.6%	63.8%	115
68	37.6%	5.0%	57.4%	128
69	29.6%	7.8%	62.6%	99
70	41.9%	5.3%	52.8%	137
71	39.6%	5.3%	55.1%	134
72	36.0%	3.2%	60.8%	122
73	39.5%	5.5%	55.0%	133
74	35.3%	4.0%	60.7%	117
75	22.3%	0.6%	77.1%	69
76	23.3%	1.6%	75.2%	76
77	22.7%	2.2%	75.1%	71
78	24.7%	2.4%	72.9%	81
79	20.5%	1.1%	78.4%	65
80	41.3%	8.8%	49.9%	138
81	44.2%	7.5%	48.3%	148
82	46.2%	7.7%	46.0%	163
83	37.1%	4.5%	58.4%	126
84	36.3%	3.2%	60.5%	119
85	30.5%	2.4%	67.1%	98
86	24.6%	2.0%	73.5%	85
87	20.9%	1.2%	77.9%	66
88	14.4%	0.9%	84.7%	44
89	27.4%	2.5%	70.1%	84
90	36.1%	4.4%	59.5%	120
91	36.0%	7.3%	56.7%	119
92	38.7%	4.5%	56.8%	127
93	21.7%	1.2%	77.1%	73
94	32.1%	3.7%	64.3%	102
95	23.9%	1.5%	74.6%	81
96	32.1%	3.1%	64.8%	112
97	33.5%	4.7%	61.8%	114
98	32.3%	9.8%	57.9%	111
99	30.0%	2.4%	67.6%	108

Table 13. Statistics of Tigecycline impact.

Image number	Cells	Matrix	Cells-free zone	Number of cells
0	46.0%	8.3%	45.6%	160
1	31.1%	3.6%	65.3%	92
2	10.5%	0.5%	89.0%	37
3	10.5%	0.8%	88.7%	33
4	16.2%	1.1%	82.7%	58
5	7.3%	0.3%	92.4%	23
6	30.9%	2.2%	66.9%	99
7	15.7%	0.6%	83.7%	50
8	4.4%	0.3%	95.4%	13
9	8.2%	0.2%	91.5%	27
10	47.8%	7.9%	44.3%	159
11	46.1%	5.7%	48.2%	137
12	6.4%	0.4%	93.2%	18
13	10.4%	0.1%	89.4%	33
14	18.1%	2.3%	79.5%	54
15	13.3%	0.3%	86.3%	40
16	44.4%	9.3%	46.3%	135
17	40.3%	12.6%	47.1%	120
18	16.0%	0.4%	83.5%	50
19	24.7%	1.2%	74.1%	85
20	50.6%	14.6%	34.7%	167
21	48.6%	9.0%	42.4%	156
22	40.2%	6.4%	53.4%	132
23	9.8%	0.2%	90.0%	33
24	7.1%	0.4%	92.5%	23
25	14.8%	0.6%	84.6%	42
26	44.8%	7.2%	48.1%	144
27	46.9%	11.4%	41.8%	151
28	44.7%	7.5%	47.8%	142
29	43.5%	7.2%	49.2%	136
30	46.9%	8.8%	44.4%	158
31	50.5%	13.3%	36.2%	167
32	46.6%	9.2%	44.2%	159
33	13.8%	0.4%	85.8%	41
34	42.7%	7.7%	49.7%	135
35	44.7%	6.5%	48.8%	138
36	48.1%	8.0%	43.9%	157
37	46.4%	10.7%	42.9%	149
38	47.1%	7.3%	45.6%	149
39	46.5%	9.0%	44.5%	141
40	47.0%	9.5%	43.5%	159
41	48.4%	8.6%	43.0%	163
42	47.3%	12.2%	40.6%	161

43	38.6%	4.5%	56.9%	119
44	44.5%	8.0%	47.5%	143
45	49.0%	8.8%	42.2%	161
46	46.2%	9.2%	44.6%	150
47	47.3%	9.4%	43.3%	154
48	44.3%	8.3%	47.4%	138
49	45.2%	7.3%	47.5%	136
50	49.5%	10.7%	39.8%	161
51	47.1%	10.6%	42.2%	152
52	47.5%	6.8%	45.7%	148
53	49.2%	11.2%	39.7%	161
54	46.2%	12.7%	41.1%	149
55	45.6%	9.1%	45.2%	147
56	47.3%	9.0%	43.6%	155
57	45.6%	9.0%	45.3%	151
58	45.9%	6.7%	47.5%	148
59	44.2%	8.3%	47.5%	137
60	48.1%	9.1%	42.7%	157
61	48.5%	11.1%	40.4%	159
62	48.5%	10.2%	41.3%	165
63	48.1%	13.3%	38.7%	157
64	49.6%	13.5%	36.9%	162
65	48.1%	10.7%	41.2%	160
66	49.0%	10.3%	40.7%	164
67	51.2%	10.0%	38.8%	165
68	47.2%	9.4%	43.4%	155
69	47.3%	11.9%	40.8%	147
70	48.4%	7.9%	43.7%	161
71	46.6%	11.1%	42.3%	154
72	50.1%	12.6%	37.3%	167
73	50.2%	11.2%	38.6%	165
74	52.0%	12.4%	35.6%	166
75	47.3%	8.6%	44.1%	164
76	46.7%	8.7%	44.6%	155
77	47.4%	10.1%	42.4%	158
78	46.3%	7.8%	45.9%	152
79	45.8%	9.1%	45.1%	147
80	47.4%	11.0%	41.6%	157
81	50.4%	12.8%	36.8%	170
82	48.3%	11.5%	40.2%	160
83	47.0%	11.0%	42.0%	149
84	50.4%	12.1%	37.5%	171
85	48.9%	11.8%	39.3%	152
86	46.5%	8.8%	44.8%	151
87	49.2%	10.6%	40.2%	161
88	45.9%	8.6%	45.5%	150
89	45.8%	10.3%	43.9%	153
90	48.6%	10.5%	40.9%	164

91	55.4%	15.4%	29.0%	189
92	51.3%	12.8%	35.9%	165
93	53.3%	14.5%	32.2%	181
94	56.3%	21.1%	22.3%	190
95	48.2%	9.6%	42.2%	164
96	48.5%	10.8%	40.6%	156
97	51.1%	13.6%	35.2%	172
98	48.2%	8.9%	42.9%	157
99	49.7%	11.6%	38.7%	157

4 Additional calculation details

Here, supplementary explanations of biofilm key parameter (areas of segmentation classes, number of cells, formation rate constants) estimation methodology are presented.

4.1 Area calculations

Areas were measured using segmentation predictions in the form of the matrix $H \times W$, where each pixel was labeled with a specific number, characterizing a specific segmentation class. To calculate the segmentation class area percentage on the image, the number of pixels, which were labeled as belonging to the segmentation class, was divided by the total number of pixels in the image ($H \times W$).

When biofilm at a specific stage of development was characterized by multiple images, either the stitching with further area calculation procedure was performed (e.g., in section “Image analysis of biofilms at scale” in the article) or the sequential area calculation on each image with the further median estimation of area percentages for each class (e.g., in sections “Kinetic modeling of biofilm growth”, “Automated mapping of large amounts of SEM images for the investigation of antimicrobial compound impact” in the article) was carried out.

Segmentation of images, which were used for kinetic modeling, was carried out with the network, which was additionally trained on the 15 images with only empty support. These 15 images were also used in area analysis and were manually annotated as full cell-free zones.

The absolute image area was calculated using the information about pixel length.

4.2 Cell counts

The number of cells on the image was estimated as the number of bounding boxes after watershed implementation. When biofilm at a specific stage of development was characterized by multiple images, the total number of cells was counted as the sum of cell counts for each image. The number of cells in the biofilm macroscale region was equal to 19 064, and the number of cells on other images (500 images, which were used to evaluate antibiotic impact) was equal to 55 672. The total number of cells was 74 736.

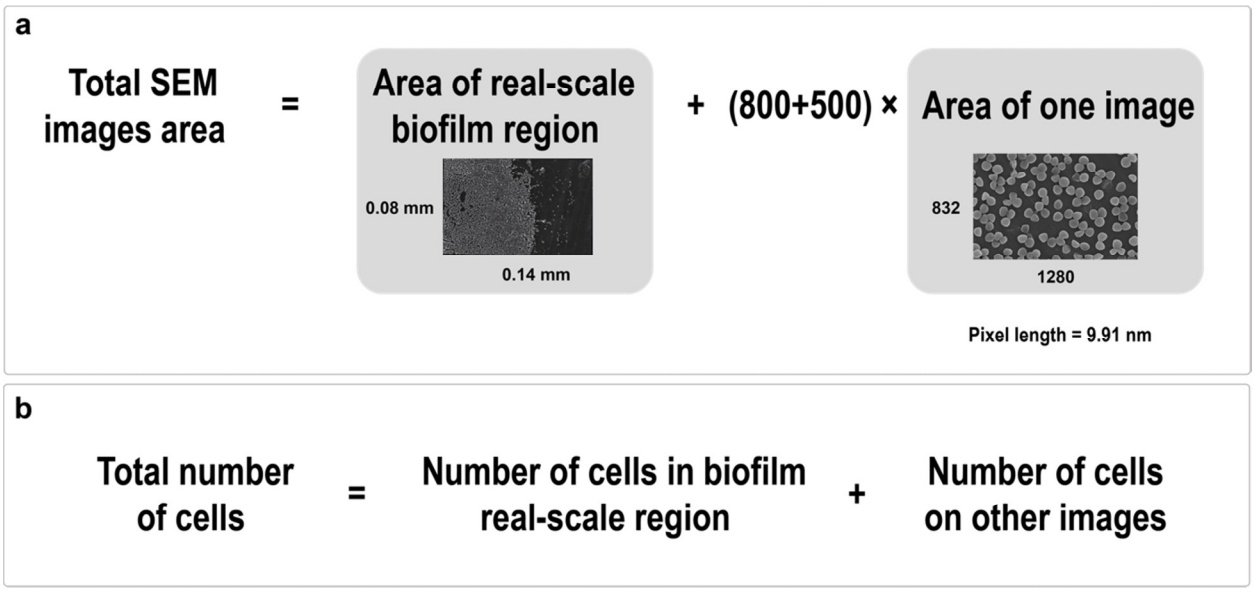

Figure 21. Formulas, which were used to estimate the amount of research. a – total SEM image area estimation. b – total number of cells estimation.

4.3 K_1 rate constant estimation

Here, we present the plot, which contains information about the rate of support coverage with cells.

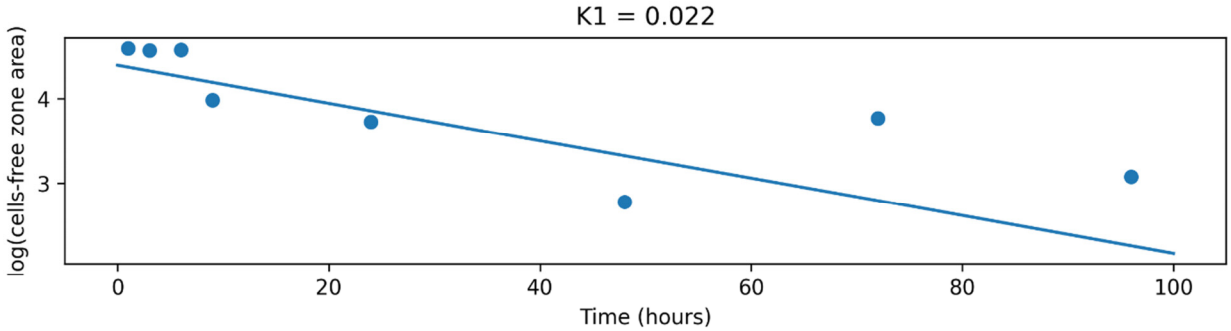

Figure 22. Linear regression between time and logarithm of cell-free zone area.

4.4 Neural network processing time

Here, we present the histogram of the neural network processing time for 400 images. The mean time is equal to 0.78 seconds.

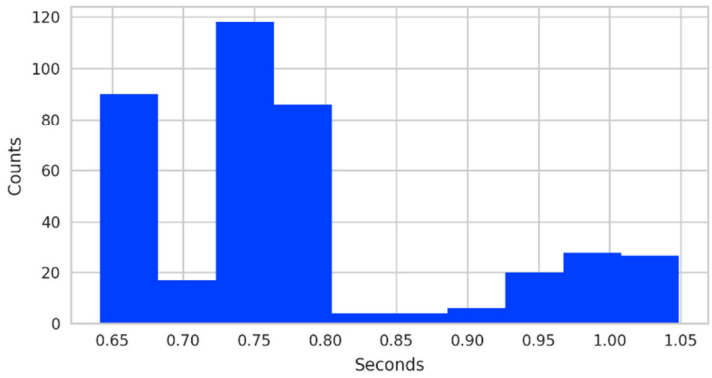

Figure 23. Model processing time distribution.

4.5 Method limitations

Restrictions and possible problems of the approach in solving applied tasks.

- It is necessary to retrain neural networks on a new annotated dataset of biofilm images if other species of bacteria are being studied.
- The difference between the results and quantitative data is due to the impossibility of reconstructing 3D images by SEM.
- Transfers, fixations and rinses can change the quantitative characteristics of biofilms. Therefore, additional validation steps are required.
- When SEM is used, it is impossible to distinguish dead cells from living cells. Therefore, the biocide effect can only be judged by an increase in the proportion of the matrix since the dead cell will be registered by the neural network as a matrix.
- We are not able to observe the change in the same sample before antibiotic treatment and after treatment, since it is necessary to inactivate and fix the biofilm.

5 Channel zone recognition

Here, an explanation of difference between channel zone and cell-free zone is shown.

Channels are observed when surface bacteria cells are in contact with each other and some space is left between them. The cells of the biofilm lower layer should be located in the area of this space. Most channels have convex shape. Channels are characterized by lower size compared to cell-free zone, which is actually an empty area of the support, where there are no biofilms. Cell-free zone is characterized by a large area, which can be of any shape. Visual differences can be seen on **Figure 24**.

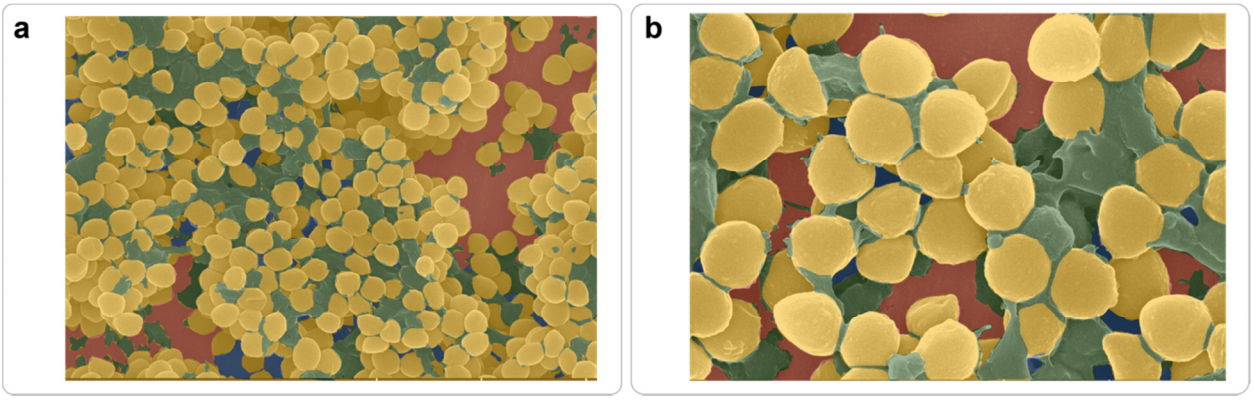

Figure 24. Annotated SEM images where the difference between channels and cell-free zone is clearly seen. (yellow — cells, green — matrix, blue — channels, red — cell-free zone). a — a big field of view; b — a small field of view.

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