Image based Machine Learning for identification of macrophage subsets

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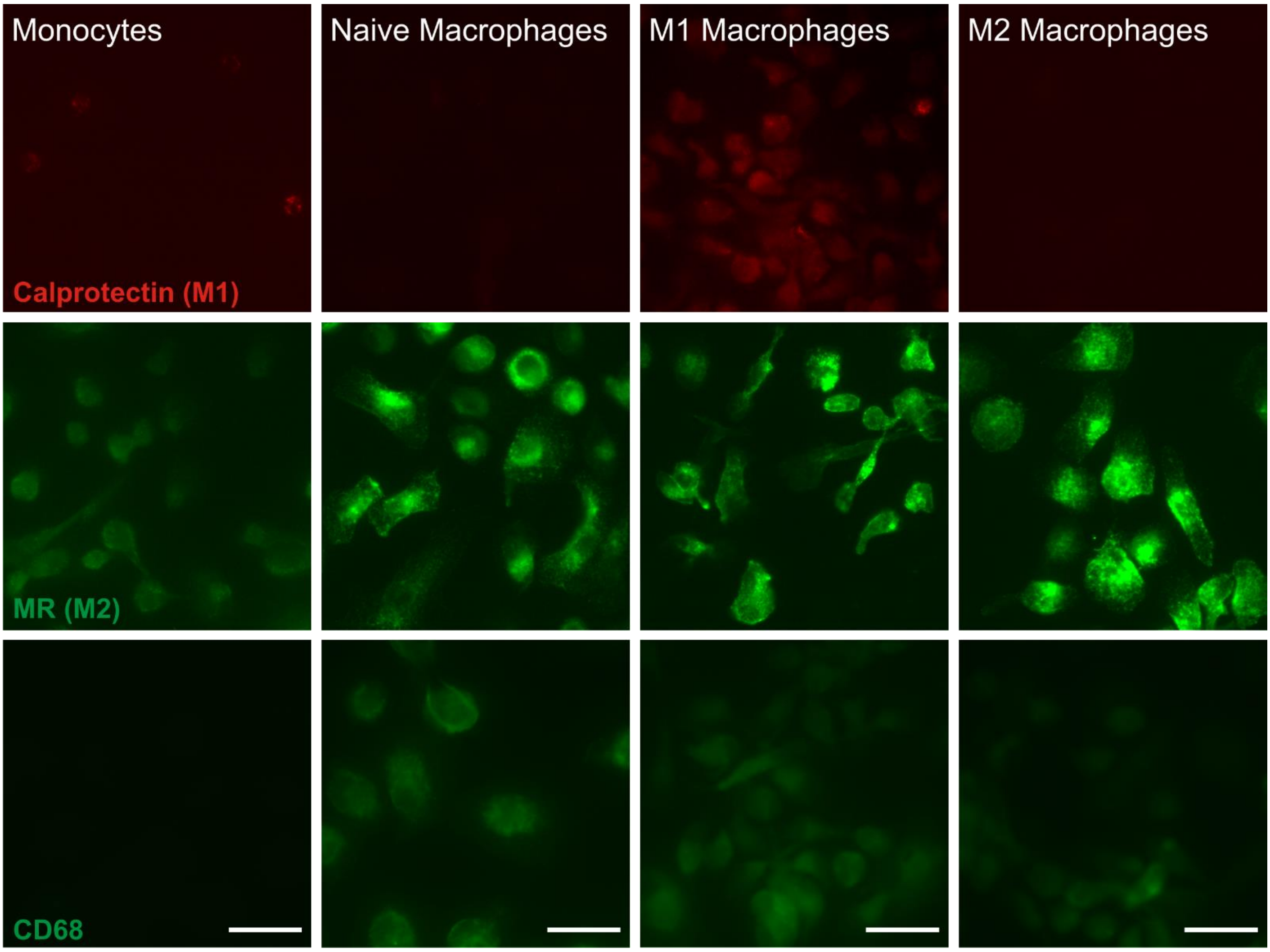
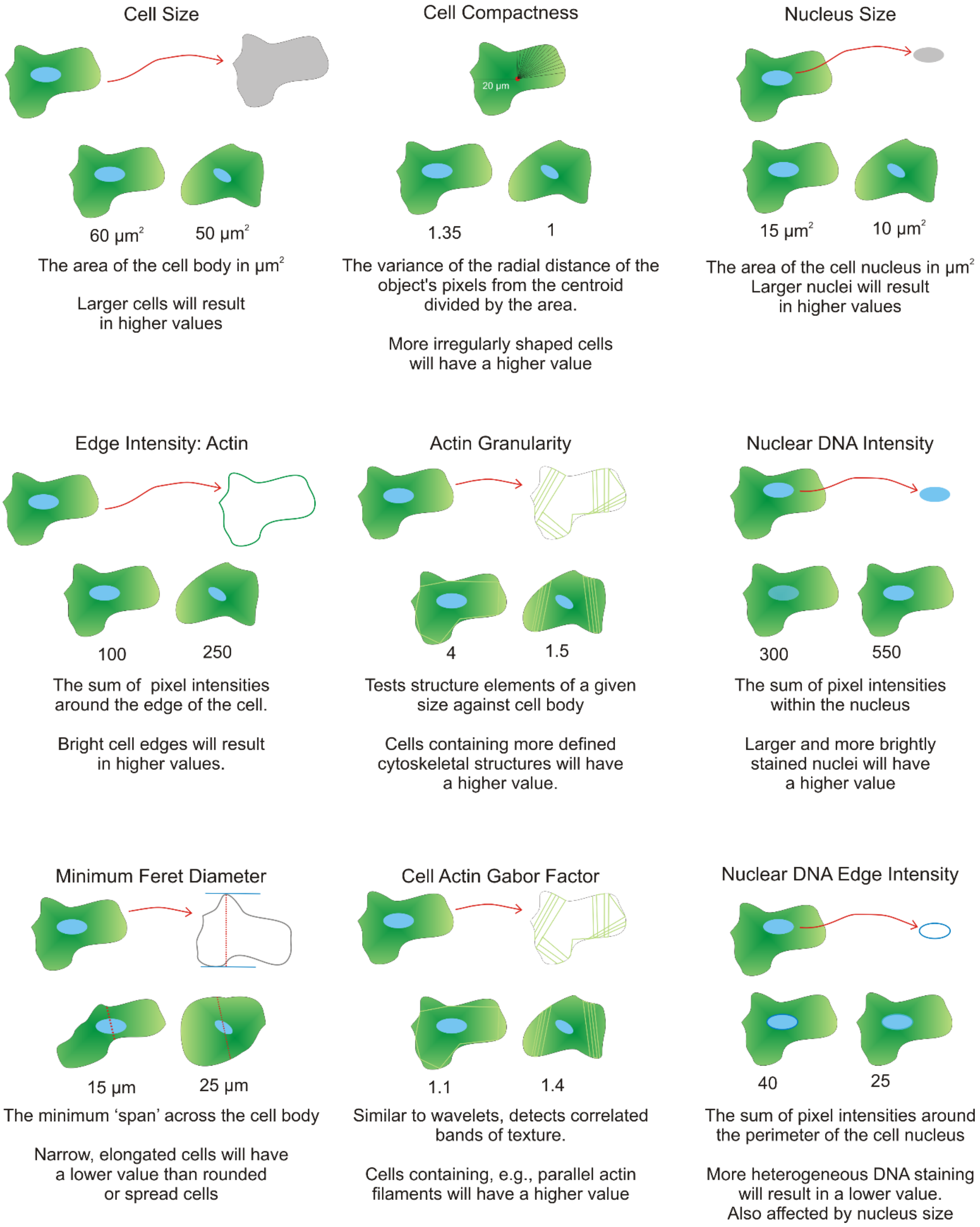


Supplementary Figure 1 Fluorescent images of cells stained for calprotectin (27E10 antigen, red), mannose receptor (MR, green), and CD68.

Scale bar = 25 µm. Representative images from n=3 are shown.

Supplementary information



Cell Size

60 µm² 50 µm²

The area of the cell body in µm²

Larger cells will result in higher values

Cell Compactness

1.35 1

The variance of the radial distance of the object's pixels from the centroid divided by the area.

More irregularly shaped cells will have a higher value

Nucleus Size

15 µm² 10 µm²

The area of the cell nucleus in µm²
Larger nuclei will result in higher values

Edge Intensity: Actin

100 250

The sum of pixel intensities around the edge of the cell.

Bright cell edges will result in higher values.

Actin Granularity

4 1.5

Tests structure elements of a given size against cell body

Cells containing more defined cytoskeletal structures will have a higher value.

Nuclear DNA Intensity

300 550

The sum of pixel intensities within the nucleus

Larger and more brightly stained nuclei will have a higher value

Minimum Feret Diameter

15 µm 25 µm

The minimum 'span' across the cell body

Narrow, elongated cells will have a lower value than rounded or spread cells

Cell Actin Gabor Factor

1.1 1.4

Similar to wavelets, detects correlated bands of texture.

Cells containing, e.g., parallel actin filaments will have a higher value

Nuclear DNA Edge Intensity

40 25

The sum of pixel intensities around the perimeter of the cell nucleus

More heterogeneous DNA staining will result in a lower value.
Also affected by nucleus size

Supplementary Figure 2 Measurements used to form the cytoprofile, as plotted in figure 5 of the main text.

Logistic Regression

	M1 Macrophage	M2 Macrophage	Naïve Macrophage	Monocyte D0	Monocyte D6	∑
M1 Macrophage	98.10%	1.40%	0.00%	0.40%	0.00%	**1560**
M2 Macrophage	2.40%	94.90%	0.00%	1.50%	1.30%	**1010**
Naïve Macrophage	0.00%	0.00%	96.60%	0.10%	3.20%	**1940**
Monocyte D0	0.00%	0.50%	0.00%	95.30%	4.20%	**660**
Monocyte D6	0.00%	3.30%	11.20%	2.70%	82.80%	**950**
∑	**1555**	**1014**	**1981**	**679**	**891**	**6120**

AUC	0.963
CA	0.944
F1	0.944
Precision	0.944
Recall	0.944

Random Forest

	M1 Macrophage	M2 Macrophage	Naïve Macrophage	Monocyte D0	Monocyte D6	∑
M1 Macrophage	92.60%	1.70%	1.60%	2.10%	2.10%	**1560**
M2 Macrophage	7.00%	85.30%	5.80%	0.80%	1.00%	**1010**
Naïve Macrophage	0.90%	0.40%	96.80%	0.20%	1.80%	**1940**
Monocyte D0	7.60%	0.50%	2.10%	89.20%	0.60%	**660**
Monocyte D6	8.50%	5.10%	16.30%	2.20%	67.90%	**950**
∑	**1663**	**947**	**2130**	**654**	**726**	**6120**

AUC	0.921
CA	0.885
F1	0.882
Precision	0.886
Recall	0.885

Naïve Bayes

	M1 Macrophage	M2 Macrophage	Naïve Macrophage	Monocyte D0	Monocyte D6	∑
M1 Macrophage	78.40%	4.00%	3.10%	7.00%	7.50%	**1560**
M2 Macrophage	10.50%	78.50%	4.50%	4.90%	1.70%	**1010**
Naïve Macrophage	3.90%	7.30%	64.60%	4.30%	19.90%	**1940**
Monocyte D0	5.00%	0.30%	0.80%	93.30%	0.60%	**660**
Monocyte D6	6.50%	6.60%	13.10%	4.40%	69.40%	**950**
∑	**1500**	**1061**	**1477**	**899**	**1183**	**6120**

AUC	0.843
CA	0.743
F1	0.744
Precision	0.761
Recall	0.743

SVM

	M1 Macrophage	M2 Macrophage	Naïve Macrophage	Monocyte D0	Monocyte D6	∑
M1 Macrophage	64.10%	13.50%	6.00%	6.90%	9.50%	**1560**
M2 Macrophage	7.40%	75.60%	10.40%	0.40%	6.10%	**1010**
Naïve Macrophage	3.50%	13.60%	63.50%	1.30%	18.10%	**1940**
Monocyte D0	2.60%	2.00%	1.80%	92.10%	1.50%	**660**
Monocyte D6	7.80%	10.30%	16.50%	2.60%	62.70%	**950**
∑	**1234**	**1349**	**1600**	**770**	**1167**	**6120**

AUC	0.806
CA	0.686
F1	0.689
Precision	0.709
Recall	0.686

kNN

	M1 Macrophage	M2 Macrophage	Naïve Macrophage	Monocyte D0	Monocyte D6	∑
M1 Macrophage	64.80%	3.80%	15.50%	9.40%	6.50%	**1560**
M2 Macrophage	23.60%	44.50%	24.30%	4.70%	3.10%	**1010**
Naïve Macrophage	27.40%	10.20%	48.50%	4.80%	9.00%	**1940**
Monocyte D0	14.70%	1.70%	7.40%	75.50%	0.80%	**660**
Monocyte D6	25.10%	11.10%	41.30%	3.80%	18.80%	**950**
∑	**2116**	**822**	**1869**	**821**	**492**	**6120**

AUC	0.674
CA	0.503
F1	0.489
Precision	0.493
Recall	0.503

Supplementary Table 1 Comparison of 5 classifier models used to segment immunofluorescent data. Confusion matrices are presented for each classifier, alongside accuracy metrics AUC (area under curve), CA (classifier accuracy), F1 (F score), Precision and Recall. All metrics are a result of 10 fold cross validation analysis.

Algorithm	Parameter settings
Logistic regression	**Regularization:** Ridge (L2), C=1
Random forest	**Number of trees:** 20 **Maximal number of considered features:** 5 **Maximal tree depth:** unlimited **Stop splitting nodes with maximum instances:** 5
Naïve Bayes	-
SVM	**SVM type:** C-SVM, C=1.0 **Kernel:** RBF, $\exp(-1.0\|x-y\|^2)$ Numerical tolerance: 0.001 **Iteration limit:** 100
kNN	**Number of neighbours:** 10 **Metric:** Euclidean **Weight:** Uniform

Supplementary Table 2 Details of classifiers used for segmentation of immune cells