Supplementary Information

Note 1. Derivation of the relation between enrichment ratio, initial target population, and classifier performance.

The enrichment ratio E is defined by

$$E = \frac{y}{x},$$

where x and y are the initial target cell proportion and the proportion of target cells after enrichment, respectively. The proportion after enrichment y can be calculated by

$$y = \frac{TPR\,x}{TPR\,x + FPR(1-x)},$$

where TPR and FPR are the true positive rate and false positive rate of a given classifier, respectively. By substituting y, the enrichment ratio E can be expressed as

$$E = \frac{TPR\,x}{TPR\,x + FPR(1-x)}\,\frac{1}{x}$$
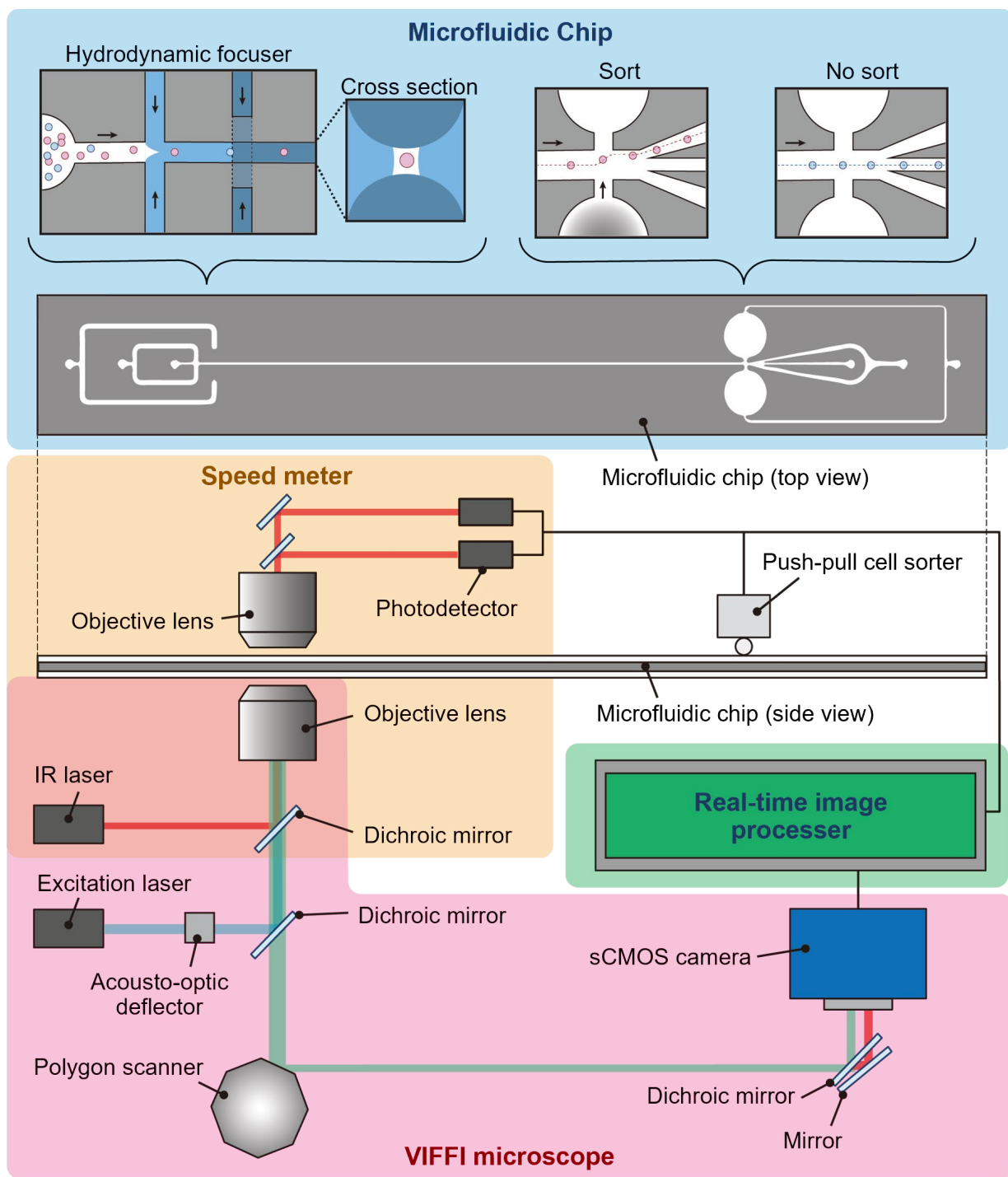
$$= \frac{1}{x + \frac{FPR}{TPR}(1-x)}.$$

Fig. S1. Simplified schematic of the iIACS 2.0 system. The four main parts of the iIACS 2.0 system[23] are depicted: the microfluidic chip (blue), the speed meter (orange), the virtual-freezing fluorescence imaging (VIFFI)[17] microscope (pink), and the real-time image processer (green). sCMOS, scientific complementary metal-oxide semiconductor; IR, infrared.
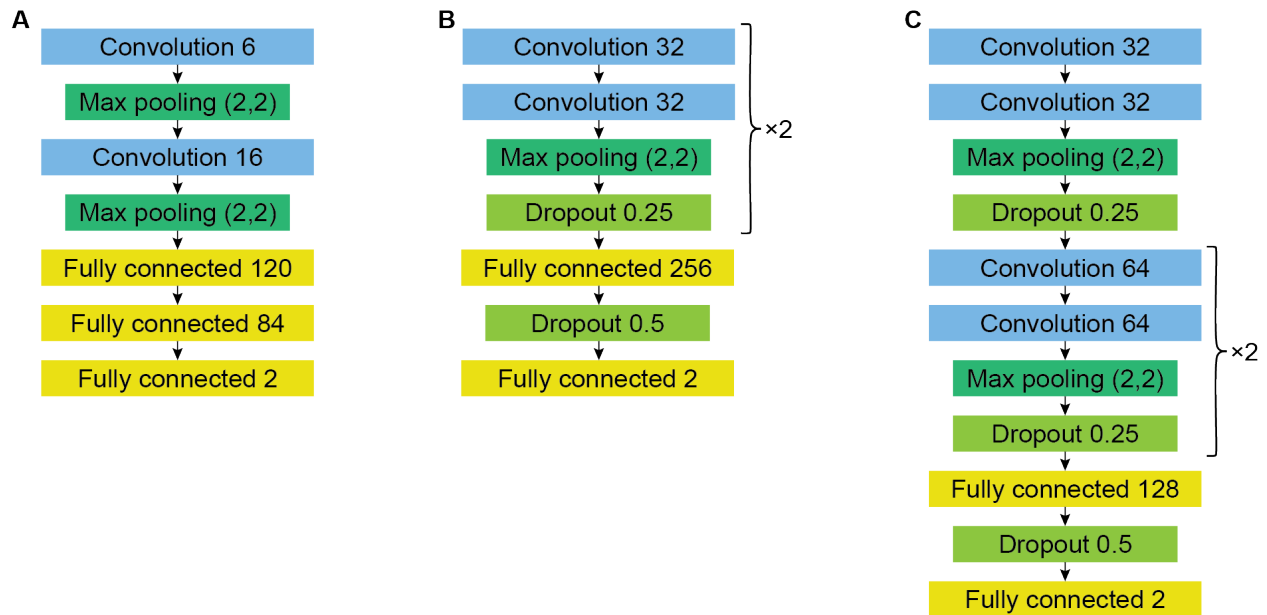
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Fig. S2. CNN structure. (**A**) Structure of the LeNet-5 classifier.[48] (**B**) Structure of the DeepCNN-6 classifier.[21] (**C**) Structure of the DeepCNN-8 classifier.[21]
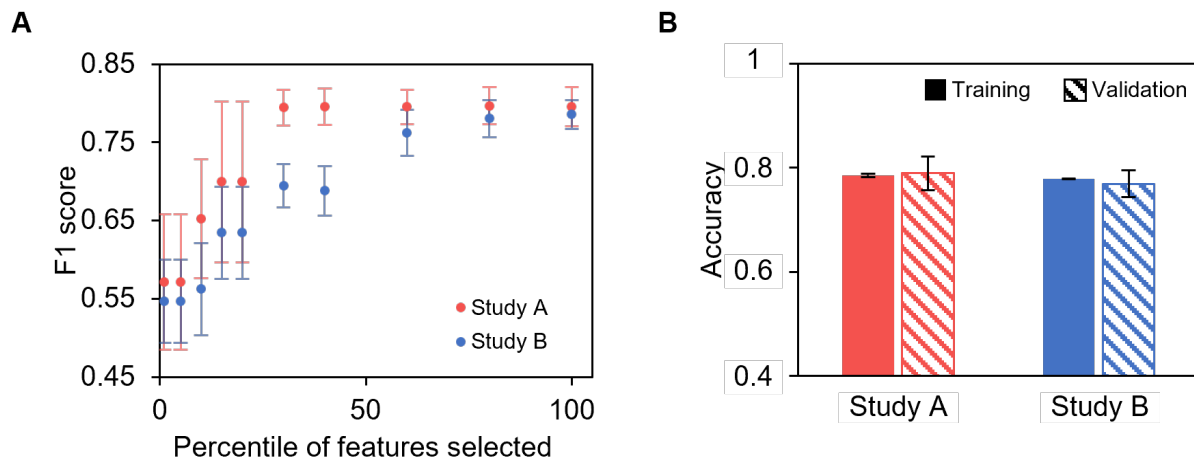
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Fig. S3. ML classifier training and validation accuracies and feature number dependence. (A) Average F1 scores of the optimized NuSVC trained on various percentiles of features selected based on analysis of variance (ANOVA) F-values. Models were trained with 5-fold cross validation. **(B)** Average training and validation accuracies of the optimized NuSVCs trained on full feature datasets with 5-fold cross validation.

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Table S1. List of extracted features and respective importance rankings. Descriptions of all features are available in CellProfiler[39] documentation for the MeasureObjectSizeShape module.

Feature name	Brief description	Study A importance ranking	Study B importance ranking
Area	The number of pixels in the object	0.00435	0.00290
Perimeter	The number of pixels around the boundary of each object	0.00701	0.00548
MajorAxisLength	The length (pixels) of the major axis of an ellipse with the same normalized second central moments as the object	0.109	0.0847
MinorAxisLength	The length (pixels) of the minor axis of an ellipse with the same normalized second central moments as the object	0.327	0.00188
Eccentricity	The ratio of the distance between the foci and its major axis length of an ellipse with the same second moments as the object	0.325	0.0266
BoundingBoxArea	The area of a box containing the object	0.00527	0.0107
FormFactor	A measure of object circularity where a value of 1 denotes a perfectly circular object	0.0208	0.00151
Extent	The proportion of the pixels in a box containing the object that are also within the object	0.0326	0.00649
Solidity	The proportion of the pixels in the convex hull of an object that are also within the object	0.0201	0.0537
Compactness	A measure of object deformation where a filled circle would have a value of 1 and irregular objects would have values greater than 1	0.0198	0.00801
MaximumRadius	The maximum distance between any pixel within the object to the closest pixel outside of the object	0.0763	0.0179
MeanRadius	The mean distance of any pixel within the object to the closest pixel outside of the object	0.0262	0.708
ConvexArea	The number of pixels in the convex hull of an object	0.00613	0.00184
MinFeretDiameter	The minimum distance between two parallel lines tangent drawn on either side of the object along all possible angles	0.00871	0.0589
MaxFeretDiameter	The maximum distance between two parallel lines tangent drawn on either side of the object along all possible angles	0.00983	0.00907
EquivalentDiameter	The diameter of a circle with the same area as the object	0.00148	0.00210