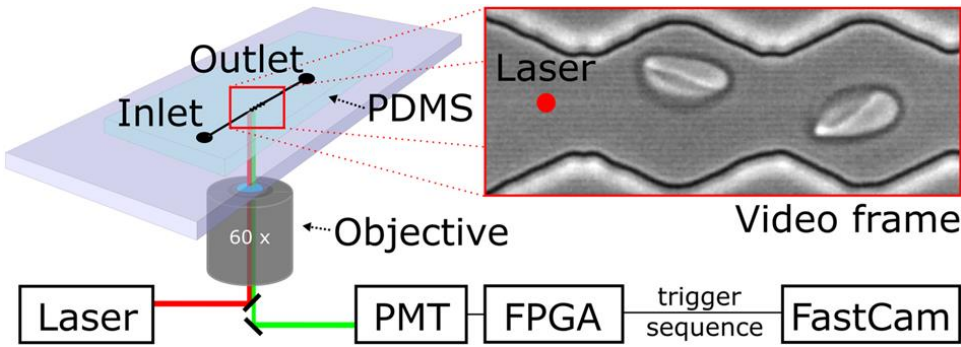


Abstract: We classify native and chemically modified red blood cells with an AI based image and video classifier. Using TensorFlow video analysis enables us to capture not only the morphology of the cell but also the trajectories of motion of individual red blood cells and their dynamics. We chemically modify cells in three different ways to model pathological conditions and obtain classification accuracies for all three classification tasks of more than 90% between native and modified cells. Unlike standard cytometers that are based on immunophenotyping our microfluidic erythrocytometer allows to rapidly categorize cells without any fluorescence labels simply by analysing the shape and flow of red blood cells.

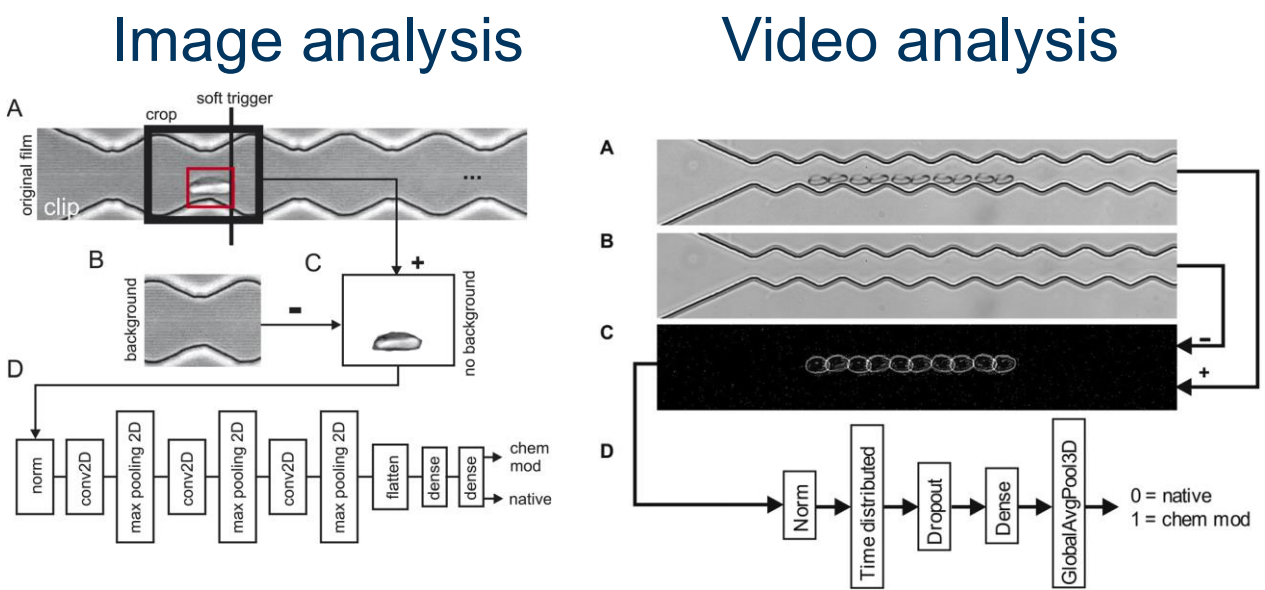
Microfluidic setup

Red blood cells (RBC) entering the device mounted on an inverted microscope and flow into an oscillating section where the RBCs continuously adopt their shape according to the channel shape and fluid flow field. Short video sequences are captured using a high-speed camera.



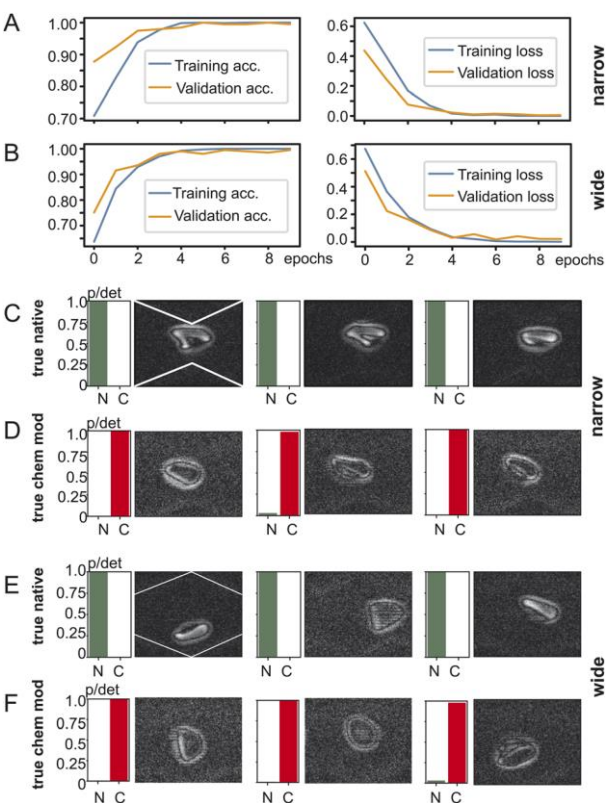
Data processing pipeline

Micrographs and data processing pipeline for image and video analysis. **(left)** (A) Single frame of a cell in the crop area was “soft-triggered” from the video sequence. (B,C) Background subtraction (D) TensorFlow layers with binary output (detection probability): (RGB value normalisation to 0.01) **(right)** (A) overlay of frames captured from video (B,C) OpenCV library employed to perform background subtraction, resulting in a bitwise pixel overlay that displays only the moving red blood cell. (D) TensorFlow layers: ‘Norm’: rescaling values (0 and 1), ‘Time Distributed’: transformation on each frame in a video batch using EfficientNetB0 base model, ‘Dropout’: standard layer for preventing overfitting, ‘dense’: standard dense layer, ‘GlobalAvgPool3D’: standard 3D average pooling layer. The final layer generates two outputs: detection probability of a native RBC and another for the chemically modified RBCs.

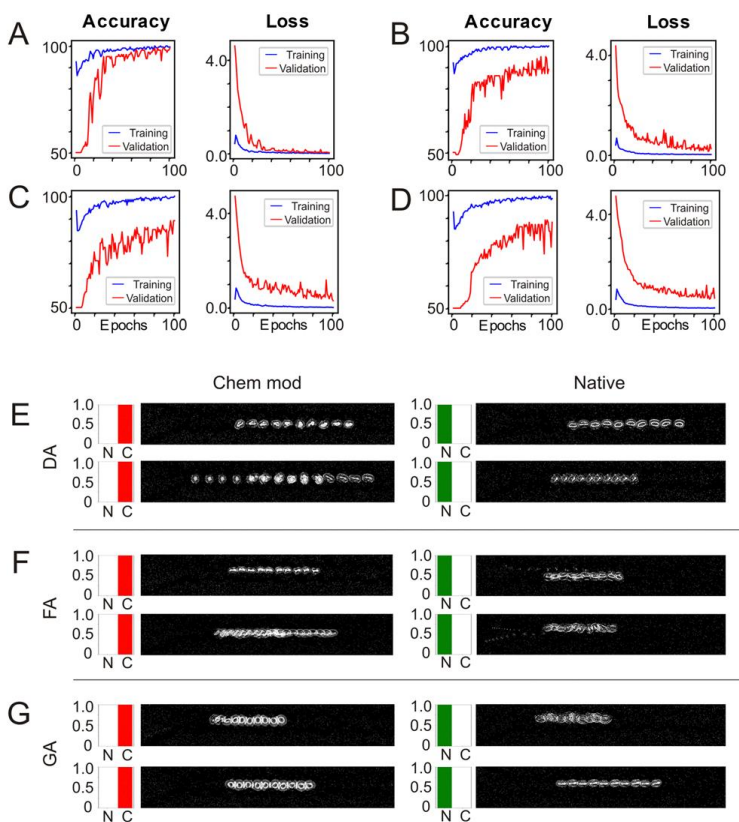


Model training and results

TensorFlow training behaviour and results. (A) Training and validation loss for the narrow part of the microchannel and for the wide part (B) of the microchannel. Classification results for the narrow section of the microchannel for (C) native red blood cells and (D) chemically modified red blood cells. Classification results for the wide section of the microchannel for (E) native red blood cells and (F) chemically modified red blood cells. “N”: native, “C”: chemically modified, “p/det”: detection probability.



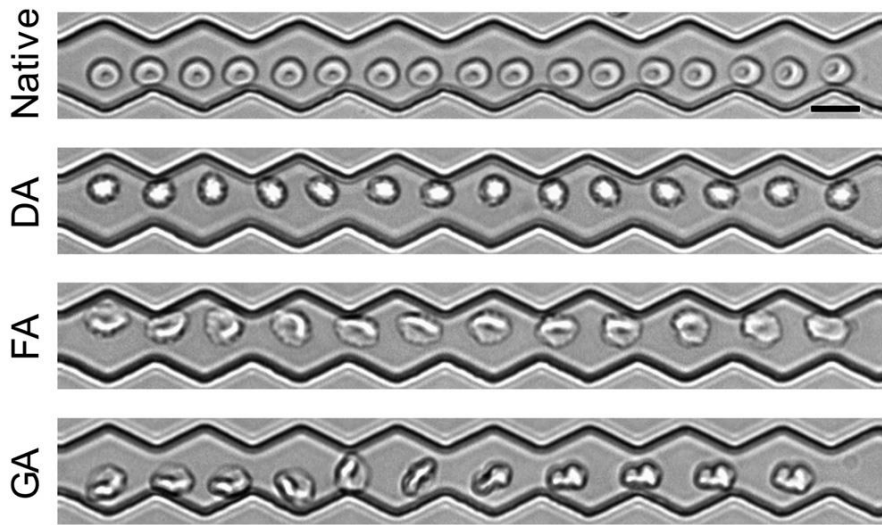
TensorFlow training and results. The line charts show the training and validation accuracy as well as the loss graphs for different datasets: native vs. DA dataset (A), native vs. FA dataset (B), native vs. GA dataset (C), and native vs. a mix of all modified datasets (D). Two classification results are shown for each batch for DA vs. native (E), FA vs. native (F), and GA vs. native (G). “N”: native, “C”: chemically modified. The images shown are superimpositions of the video frames for better visualization and illustration.



Micrographs of representative video sequences

Native RBC and three different chemically modified RBC (DA: diamide, FA: formalin, GA: Glutaraldehyde). Native RBC show moderate cell deformation with tank-treading motion. Spiked RBCs with rough echinocyte-like surface (DA and FA), and wrinkled surface and strongly deformed RBC with tumbling motion (GA).

	Training (%)	Validation (%)	Test (%)
DA	99.5	99	98
FA	100	89	93
GA	100	89	93
Mix	98.5	88	87



Conclusion

Two different approaches are presented to classify native and chemically modified RBC. The image-based AI analysis compares the morphological appearance of RBC at two defined sections in the oscillating microchannel, the narrow and the wide part, whereas the video classification AI additionally incorporates the motion of the RBCs. These end-to-end classification for image or video-based AI are capable to distinguish the different cells with high accuracy.