Electronic Supplementary Information (ESI)

for

Human Blood Platelets Contract in Perpendicular Direction to Shear Flow

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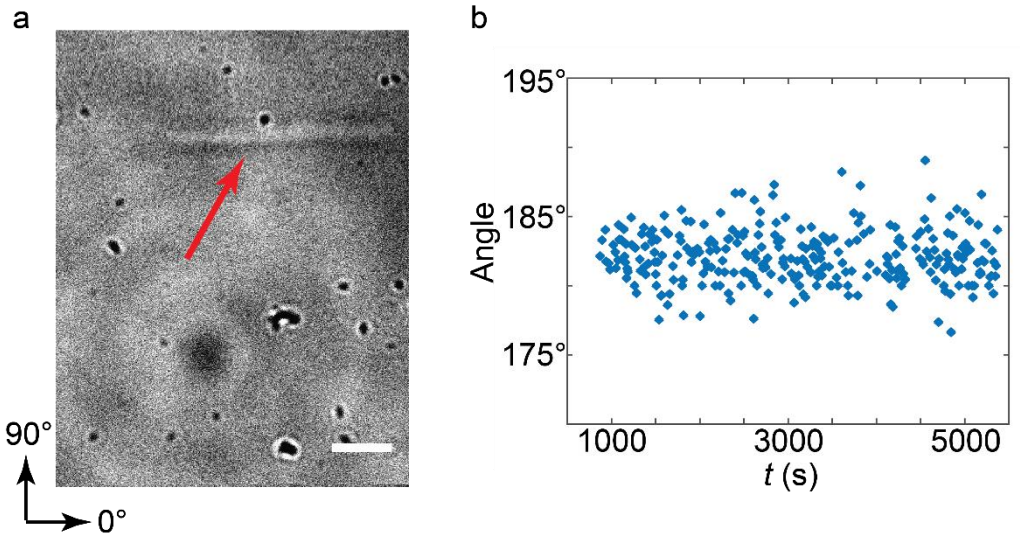
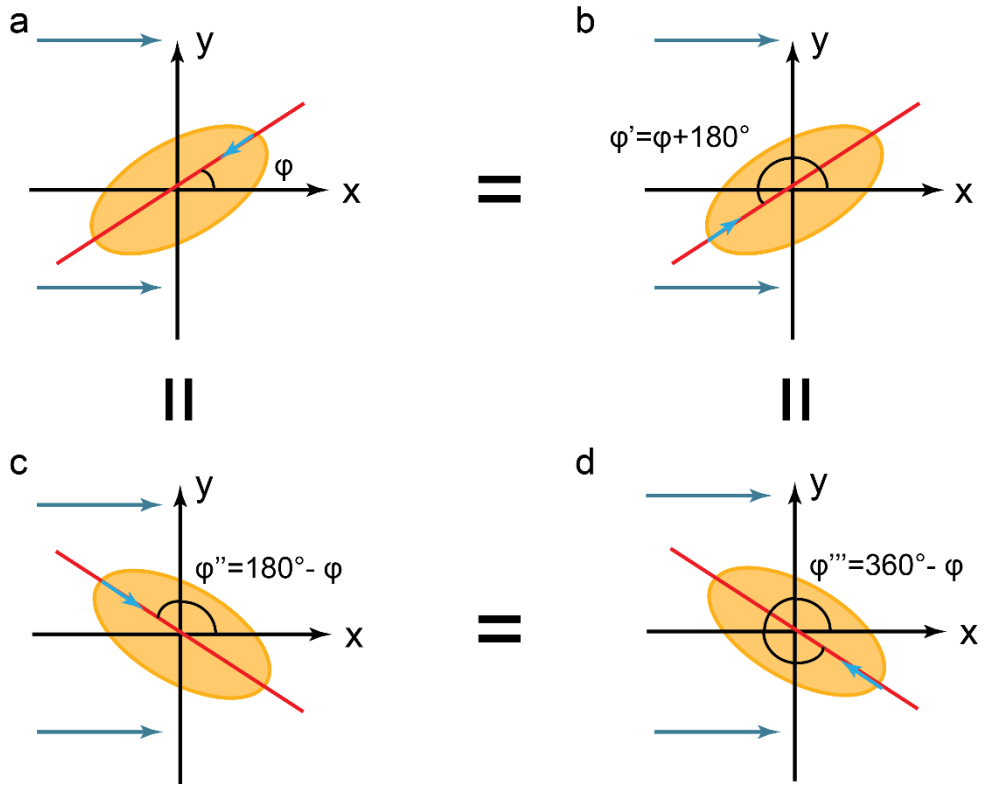
1. Measurement of the Flow Direction



Supplementary Figure S1 a) The direction of the flow is determined by the traces of platelets passing by (see red arrow for an example). The angle of the flow direction is given as indicated below the image. In this example, the flow points from right to left, resulting in an expected flow angle at about 180°. Scale bar: 5 µm. b) Temporal development of the angle. The flow direction is stable over time within the error bounds of 10 % of the individual measurements. This measurement corresponds to a recording at a flow rate of 300 µL/h.

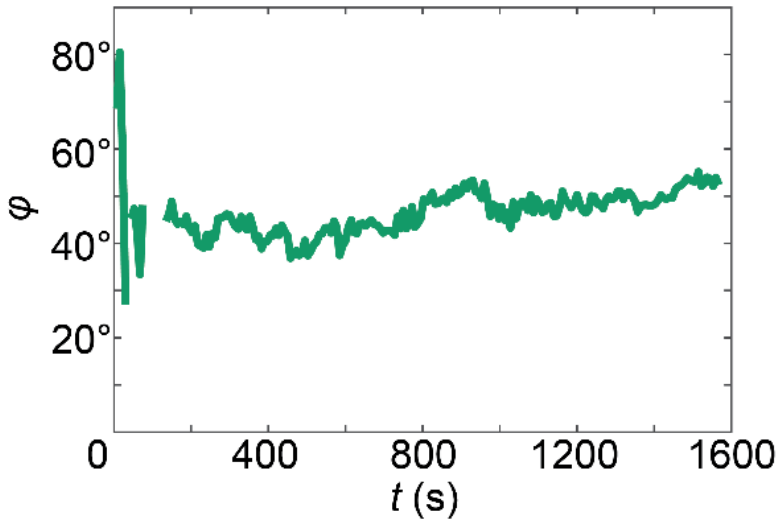
2. Determination of the Contraction Angle

2.1. Possible Angles



Supplementary Figure S2 Angles between flow direction and major dipole axis that are considered the same. The platelet is shown as an orange ellipse, the red line denotes the major dipole axis. Here, the flow (blue arrows) points along the positive x-axis. The light blue arrow marks the determined dipole vector pointing inwards towards the center of the cell. a) Definition of the angle φ between the flow and the dipole axis. b) Due to the nature of the dipole axis, the angles φ and $\varphi + 180°$ are identical, describing the same eigenvector with opposite sign. c) Angles of φ and $180° - \varphi$ as well as d) $360° - \varphi$ also are considered identical. Thus, all calculated angles are found between 0° and 90°.

2.2 Temporal Development of the Dipole Angle

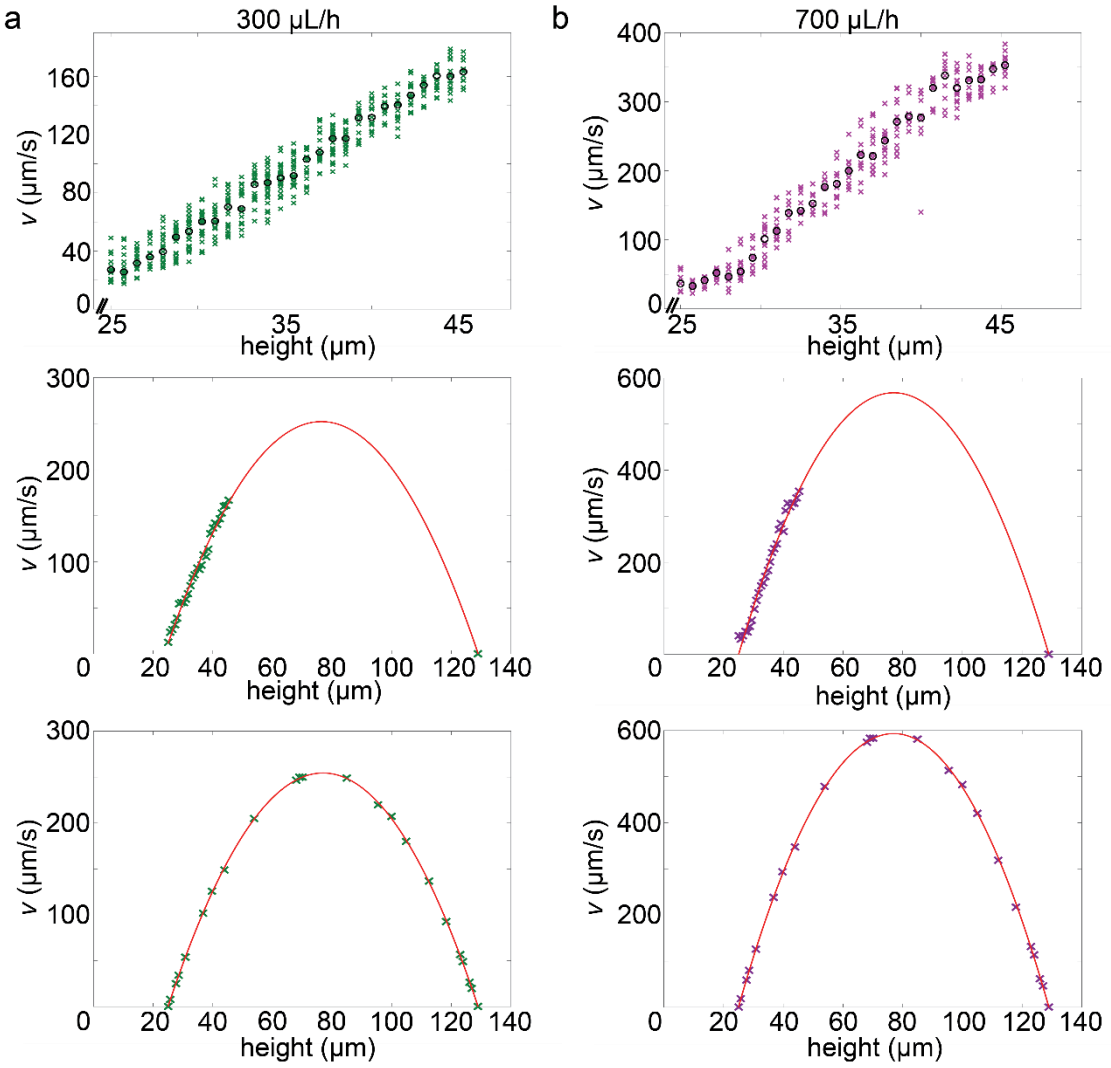


Supplementary Figure S3 Temporal development of the preferred direction of contraction. During the initial stages, when no stable force is observed, the angle fluctuates or no value can be determined at all, corresponding to an instable dipole matrix. Starting at about 150 s, the angle stabilizes as the force and dipole stabilizes and varies no more than 10 % from the average angle. This data set was recorded at a flow rate of 300 µL/h.

3. Verification of Shear Rates in Experiment and Simulation

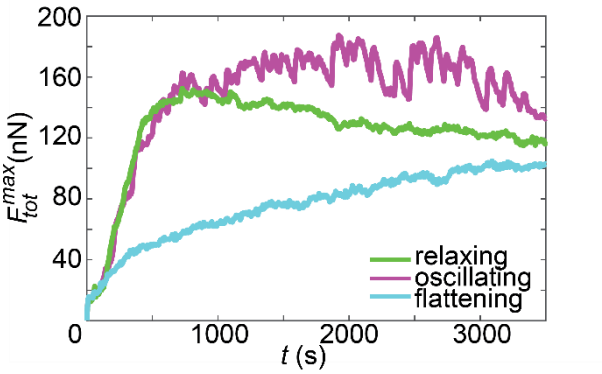
We conducted additional experiments to determine the flow profile within the measuring chamber. The microfluidic system was set up in a similar manner as in the actual experiments with the substrate-containing chamber and the mixing channel connected in series. All three syringes (Hamilton gas-tight syringes, series 100) were filled with HT-BSA buffer containing 500 nm red fluorescent beads at a dilution of 1:1999 (FluoSpheres 2% solids, carboxylate-modified microspheres, 580/605 nm, Thermo Fisher Scientific Inc., Waltham, MA, USA). The syringes were driven by syringe pumps as during the cell experiments, *i.e.* at a ratio of 1:18 between each syringe connected at a side inlet and the syringe at the central inlet. All total flow rates (300 µL/h, 500 µL/h and 700 µL/h) were tested. The fluorescent beads in the solution were tracked by fluorescence microscopy within the measuring chamber, directly above the substrate. For details concerning the microscopy setup, please see the main text, Materials and Methods section. The illumination time was 200 ms at a lamp intensity of 100%. In total, 20 µm height could be scanned (in steps of 0.7 µm) before the beads became too fast to be tracked within the field of view. The length of the traces together with the exposure time revealed the velocity of the particles at the specific position.

At each position, at least three images were analyzed, corresponding to, on average, 10 traces. As the traces directly at the substrate surface were fewer than at higher positions and comparatively short, and thus prone to errors, additional 50 images were recorded and analyzed. This added another 200 traces at this position, which served to validate the velocity values obtained from the first recordings. The length of each traces was measured using ImageJ. The measured velocity values for the recorded positions are shown in the top row in Fig. S4. A parabolic fit was applied to the mean velocities using Matlab (see Fig. S4, center). The fits demonstrate how well the measured values fit to the expected flow profile. From this fit, the shear rates were calculated by determining the profiles' derivatives in proximity of the substrate surface. These values were compared to a flow profile determined using Comsol Multiphysics as described in the main text. Here, we assumed a laminar flow profile and solved the stationary Navier-Stokes equation (main text, Eq. (4)). The discrete velocity values were then fitted to a parabola using Matlab to determine the corresponding shear rate (Fig. S4, bottom).



Supplementary Figure S4 Starting at the gel surface at 25 µm, the flow fields in different planes for a total of 20 µm height were recorded for the total flow rates of 300 µL/h (a), 500 µL/h (not shown) and 700 µL/h (b). Top: measured velocity values (crosses) with their median (circles). Center: Parabolic fit (red line) to the mean velocities (crosses). Bottom: simulated velocity profile determined using Comsol Multiphysics (crosses) and parabolic fit (red line). The difference in the maximum velocity between the simulated and measured velocity is less than 5 %.

4. Temporal Contraction Behavior of Different Platelets



Supplementary Figure S5 Examples of different contraction behaviors observed for the various flow rates. While for some platelets the force started to level off after some time (cyan), others relaxed soon after initial contraction (green). In some cases, oscillations could be observed (magenta). All force curves shown here were recorded at a flow rate of 500 µL/h.