## Supplementary Information for "Cross-sectional focusing of red blood cells in a constricted microfluidic channel"

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## **Supplementary Figures**



**Fig. S1** Microscopic snapshots of (a-c) a 5% *Ht* living RBC suspension and (d-f) a 5% *Ht* rigid RBC suspension flowing through the microfluidic contraction-expansion channel. From top to bottom the pressure drops are  $\Delta p = 40$  mbar,  $\Delta p = 100$  mbar, and  $\Delta p = 140$  mbar, respectively. The direction of flow is from left to right, as indicated by the white arrow. The white scale bars represent a length of  $100 \ \mu$ m.



**Fig. S2** Velocity of rigid RBCs (1% *Ht*) x = 10 mm post-contraction at a pressure drop of (a)  $\Delta p = 40$  mbar and (b)  $\Delta p = 140$  mbar. Cells are detected at channel center in *y* direction in two layers, close to the bottom ( $z \approx -15 \mu$ m) and in the channel middle (z = 0), represented as blue and red symbols, respectively. Dashed lines represent the mean velocities in each plane.



**Fig. S3** Thickness of the cell-free layer for rigid and living RBCs (5% Ht) as a function of pressure drop. The thickness is calculated x = 10 mm post-contraction. Dashed lines are to guide the eye.



**Fig. S4** Distribution of rigid RBCs (1% *Ht*) x = 10 mm post-contraction along the channel width close to the top (a), in the channel middle (b), and close to the bottom (c) in *z*-direction. The pressure drop is  $\Delta p = 140$  mbar. Data is accumulated over 4,000 frames.



**Fig. S5** Distribution of rigid RBCs (1% *Ht*) x = 10 mm post-contraction along the channel width close to the top (top row), in the channel middle (middle row), and close to the bottom (bottom row) in *z*-direction. (a), (c) and (e) represent the channel border and (b), (d) and (f) show the channel center in *y*-direction. The pressure drop is  $\Delta p = 40$  mbar. Data is accumulated over 4,000 frames.



**Fig. S6** Distribution of rigid RBCs (1% *Ht*) x = 10 mm pre-contraction along the channel width in the channel middle (upper row), and close to the bottom (lower row) in *z*-direction. (a) and (c) represent the channel border and (b) and (d) show the channel center in *y*-direction. The pressure drop is  $\Delta p = 140$  mbar. Data is accumulated over 4,000 frames.



**Fig. S7** PTV: Velocities of individual rigid RBCs (1% *Ht*), plotted as blue dots, at  $\Delta p = 140$  mbar (a) x = -10 mm pre-contraction and (b) x = 10 mm post-contraction. Data is acquired across the whole channel height, using a  $10 \times 10^{-10}$  lens with NA = 0.3. The dashed and solid red lines represent the maximum and mean velocities of all cells along the channel width, respectively. The dotted black line indicates the velocity profile for a Newtonian fluid across the channel middle z = 0.



**Fig. S8** PIV: Mean velocity profiles across the channel width at x = 10 mm post-contraction of living RBCs (5% *Ht*) at different pressure drops in a density-matched solution (35% OptiPrep<sup>TM</sup> and 65% PBS).



**Fig. S9** Time stacks of 1,050 microscopic images of (a-c) a 5% *Ht* living RBC suspension and (d-f) a 5% *Ht* rigid RBC suspension flowing through the microfluidic contraction-expansion channel. From top to bottom the pressure drops are  $\Delta p = 40$  mbar,  $\Delta p = 140$  mbar, and  $\Delta p = 240$  mbar, respectively. The direction of flow is from left to right, as indicated by the white arrow. The white scale bars represent a length of 100  $\mu$ m.