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

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Supplementary Figures

\textbf{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 ($z \approx -15$ µm) 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 $10 \times$ 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™ 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.