Supporting Information for:

Title:

Pinched-flow hydrodynamic stretching of single-cells

Authors:

Jaideep S. Dudani[1], Daniel R. Gossett[1,2,4], Henry T.K. Tse[1,2,4], Dino Di Carlo[1,2,3]

Affiliations:

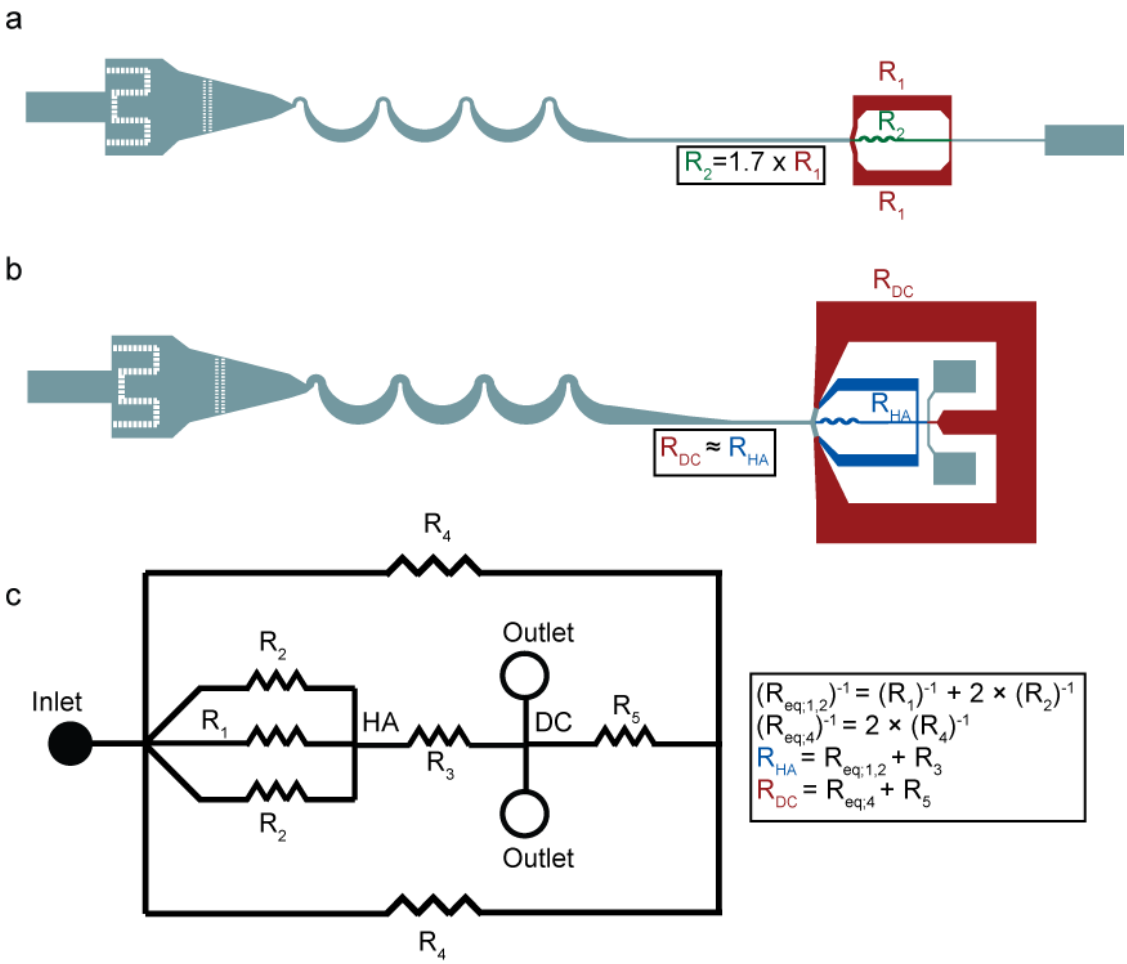
[1]Department of Bioengineering, University of California Los Angeles, Los Angeles, California 90095, USA
[2]California NanoSystems Institute, Los Angeles, California 90095, USA
[3]Jonsson Comprehensive Cancer Center, Los Angeles, California 90095, USA
[4]Present Address: CytoVale Inc., South San Francisco, California 94080, USA

[*]Corresponding Author:

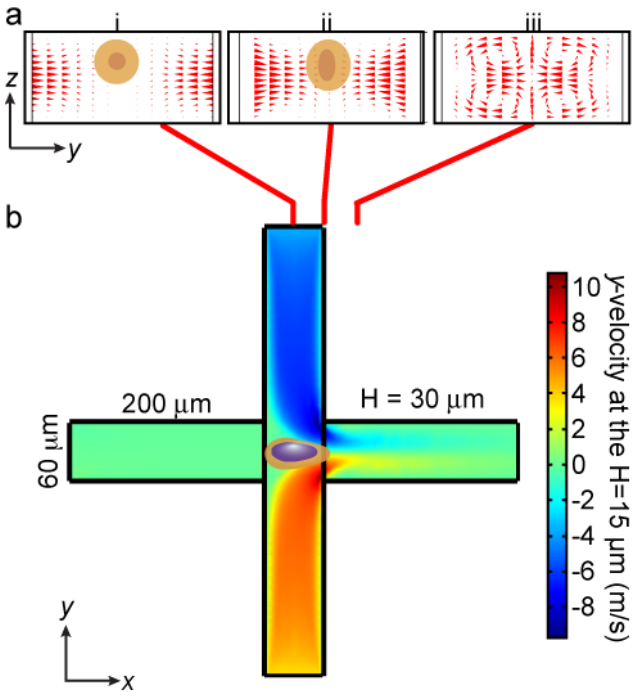
Dino Di Carlo, PhD
Associate Professor in Bioengineering
420 Westwood Plaza
5121 Engineering V, Box 951600
Los Angeles, CA 90095-1600
Email: dicarlo@seas.ucla.edu
Phone: (310) 983-3235
Fax: (310) 794-5956

Table of Contents

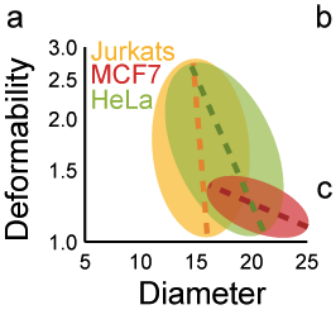
Supporting Figures



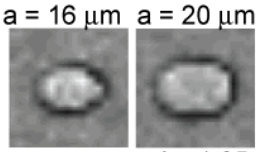
SI Figure 1. Design and resistance tuning for microfluidic devices employed. a. Computer-aided design of HA microchannels (top view) with important resistances highlighted. This leads to a decreased fraction of flow down the middle channel but allows for sufficient Reynolds number for efficient inertial focusing. The outer branches have a lower resistance to allow for a higher flow rate and velocity. This enables a higher pressure on the cells as they pass through the hydrolumen. b. Computer-aided design of combined HA-DC microchannels (top view) with important resistances highlighted. c. Simplified resistor diagram of the combined HA-DC device. Tuning of resistance is used to ensure equal flow through the two branches of channels creating the extensional flow (R_{DC} and R_{HA}).



SI Figure 2. Finite element method simulations of flow conditions. a. Velocity slices in the *yz* plane. i. As cells enter the hydropipetting region, the higher velocity flow from the branches impinge on the cell and cause it to deform due to pressure drag (ii). iii. Due to the higher pressure near the channel centerline, a secondary flow develops. b. Additionally, cells approach the hydrolumen at a low velocity compared to the squeezing flow. Formation of this hydrolumen creates a velocity gradient across the cell body – higher velocity near the cell front and lower near the rear - and a corresponding viscous drag.



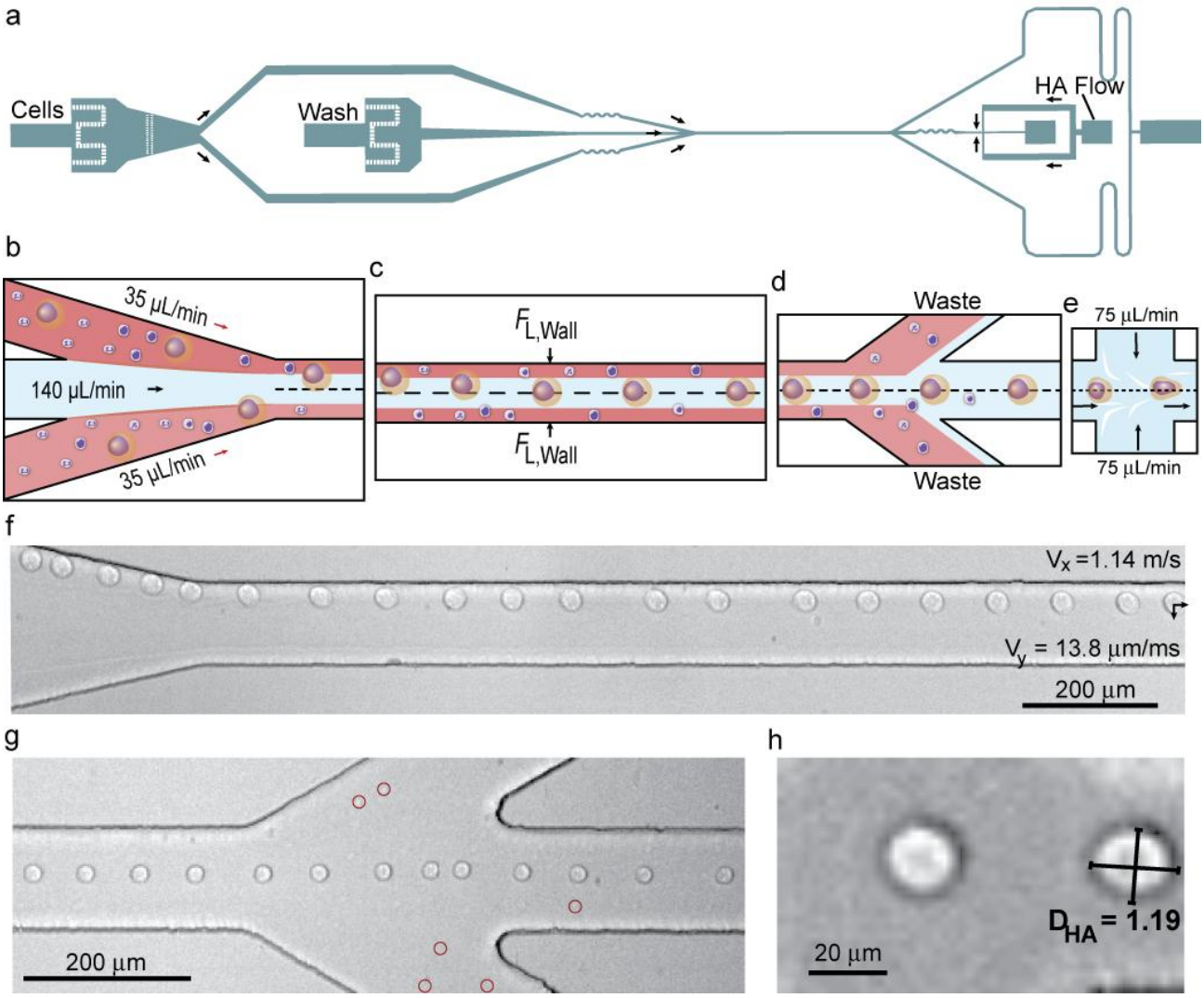
b



c

Cell Line	Fit	R^2
Jurkats	-0.047	0.98
MCF7	-0.030	0.87
HeLa	-0.038	0.96

SI Figure 3. Correlation of size with deformability. a. Deformability profiles of three different cell lines. Dotted lines indicate the slope. b. Smaller cells display a higher aspect ratio compared to larger cells. Larger cells need to deform more to achieve a high aspect ratio. c. Analysis of three cell lines reveals an negative slope (fit) of deformability with size. Slopes vary across cell lines. Deformability was analyzed after binning of cells into 5 µm diameter intervals and fitted to a linear slope.



SI Figure 4. Hydropipetting can be combined with rapid inertial solution exchange for integrated sample preparation and analysis. a. Top view of the microchannels for combined inertial solution exchange and hydropipetting. b. MCF7 breast cancer cells spiked into diluted, whole blood join a coflow of an exchange solution buffer, such as phosphate buffered saline (PBS). c. In the main channel, size-dependent lift forces act upon the larger breast cancer cells to transfer them into the wash solution. The majority of the blood cells are siphoned off to waste channels and the breast cancer cells proceed to the hydropipetting junction. d. PBS is also injected through the inlet labeled HA flow in segment e. This fluid joins the cell suspension as in hydropipetting alone. f. Overlaid high-speed images of a single breast cancer cell migrating towards the channel centerline. It travels downstream at 1.14 m/s and migrates laterally at 13.8 µm/ms. g. Breast cancer cells are enriched over the initial blood suspension, thereby decreasing the non-desired population of cells from appearing in the final measurements. Red circles indicate white blood cells. Greater than 70% of white blood cells are rejected. h. Overlaid image of a cell prior to and immediately following deformation. Cells are deformed in much the same way as hydropipetting alone with the added benefit of a decreased sample preparation time.

Supporting Video Captions

SI Video 1. Siphoning sheath fluid from the edges of an inertially concentrated flow. Inertially focused Jurkat leukemia cells are collected through the center branch of the trifurcated channel, and cell-free fluid is siphoned off in side branches. The video was captured with a 10× magnification, 0.45 NA objective at 60,768 frames per second and slowed down ~8, 600×.
Tags: Inertial Focusing, Fluid Siphoning

SI Video 2. Pinched-flow hydrodynamic stretching ("Hydropipetting"). Side branches rejoin the center branch, and siphoned sheath fluid is used to pinch the cell suspension, hydrodynamically stretching Jurkat cells. The video was captured with a 10× magnification, 0.45 NA objective at 519,083 frames per second and slowed down ~26,000×.
Tags: Pinch-flow, Hydrodynamic Stretching, Deformability, Mechanophenotyping

SI Video 3. Intermediate filament modifications in Jurkats increases deformability. Jurkat cells modified with Calyculin A exhibited an increased deformability. The video was captured with a 10× magnification, 0.45 NA objective at 519,083 frames per second and slowed down ~52,000×.
Tags: Intermediate Filaments, Deformability, Jurkats

SI Video 4. Combined Hydropipetting and Deformability Cytometry. Jurkat cells are first deformed in a pinched-flow and then in an extensional flow with a geometry similar to that of our Deformability Cytometry method. The video was captured with a 10× magnification, 0.45 NA objective at 519,083 frames per second and slowed down ~52,000×.
Tags: Hydropipetting, Deformability Cytometry, Hydrodynamic Stretching

SI Video 5. Inertial sample preparation for Hydropipetting of heterogeneous biofluids. A large MCF7 cell (breast cancer epithelial) begins to migrate out of a suspension of hypotonically lysed red blood cells, white blood cells, and spiked MCF7 cells into a clean buffer solution upstream of the hydropipetting pinch flows. The video was captured with a 10× magnification, 0.45 NA objective at 62,788 frames per second and slowed down ~6,300×.
Tags: Rapid Inertial Solution Exchange, Inertial Transfer, Automated Sample Preparation

SI Video 6. Upstream debris removal via inertial solution exchange. A MCF7 cell has migrated out of the initial suspension and is directed down a branch leading to the hydropipetting lumen while blood components are siphoned off as waste (Note: the siphoned waste is not used to pinch flow). The video was captured with a 10× magnification, 0.45 NA objective at 97,281 frames per second and slowed down ~9,700×.
Tags: Background Noise Reduction, Rapid Inertial Solution Exchange, Sample Preparation and Analysis