Supplementary Information

Inertial focusing of ellipsoid *Euglena gracilis* cells in a stepped microchannel

Ming Li^{1,2}, Hector Enrique Muñoz², Alexander Schmidt², Baoshan Guo³, Cheng Lei³, Keisuke Goda^{1,3,4}, and Dino Di Carlo^{2,5,6*} ¹Department of Electrical Engineering, University of California, Los Angeles, USA ²Department of Bioengineering, University of California, Los Angeles, USA ³Department of Chemistry, University of Tokyo, Japan ⁴Japan Science and Technology Agency, Japan ⁵California NanoSystems Institute, University of California, Los Angeles, USA ⁶Jonsson Comprehensive Cancer Centre, University of California, Los Angeles, USA ^{*} dicarlo@seas.ucla.edu



Figure S1 Histogram distribution of aspect ratios of *E. gracilis* cells (N=2,000) grown autotrophically under continuous light.



Figure S2 Characterization and measurement of cell mean intensity (CMI). (a) Schematic Illustration of 5 different imaging focal positions in vertical direction. (b) Histograms of cell mean intensity values obtained from 5 different imaging focal positions at Re= 18.5. (b) Images of *E. gracilis* cells with aspect ratio of 4 at the 4th imaging focal position. The variation of the mean intensity value is due to different vertical positions of cells.



Figure S3 Laser-two-focus (L2F) system. (a) Schematic showing L2F principles. (b) Experimental setup of integrating the stepped microchannel with L2F for applications in flow cytometry.



Figure S4 Fluorescence microscopic images in FITC channel showing chlorophyll autofluorescence in *E. gracilis* cells. Scale bars represent 20 μ m.

Supporting Movie Captions

Supplemental Movie S1

E. gracilis single-stream focusing. A high speed microscopic video of *E. gracilis* flowing through the stepped channel at inlet, #20 step and outlet at *Re*= 92.6.