Supplementary Information

Wide-field imaging and flow cytometric analysis of cancer cells in blood by fluorescent nanodiamond labeling and time gating

Y. Y. Hui, L.-J. Su, O. Y. Chen, Y.-T. Chen, T.-M. Liu, and H.-C. Chang

Wide-field low cytometric analysis. A MATLAB-based program[1] that automatically processes video signals has been developed to identify and quantify FND-labeled cells. To begin the post-processing, we use the Andor Solis program to export video data (**Figure S1a**) as a series of matrixes, each representing one frame (picture) in the video. The matrix elements are 4-digit numbers indicating the intensity of the corresponding pixel. To process each frame (matrix), an image smoothing technique known as "Gaussian blur"[2,3] is first applied to reduce image noise and details (**Figure S1b**). Given an intensity threshold, the program creates a binary bit map, assigning each corresponding element 1 (if above the threshold) or 0 (if below the threshold) (**Figure S1c**). The program then identifies and enumerates the islands of 1's in a sea of 0's (**Figure S1d**). Finally, the program calculates the approximate coordinates and records the location (coordinate) and time (frame number) for the individual islands which are now potential candidates of the FND-labeled cell signals (**Figure S1e**).

To vastly reduce algorithm complexity without compromising too much accuracy, we make 2 major assumptions: 1) cell sizes must fall within a narrow range and 2) cells travel with a constant velocity. Based on the first assumption, our program eliminates unrealistically small and large islands. The second assumption suggests that signals belonging to one specific cell must appear in consecutive frames and must be aligned in a linear fashion (**Figure S2**). Therefore, spots capable of being fitted into a straight line in the location versus time graph should then be grouped together and counted as one cell signal. The program iterates the above process and identifies all cells in the video and records information such as average intensities and velocities of the individual cells.

References:

1. http://www.mathworks.com/help/images/ref/fspecial.html
2. http://homepages.inf.ed.ac.uk/rbf/HIPR2/gsmooth.htm
3. http://rsbweb.nih.gov/ij/docs/guide/146-29.html

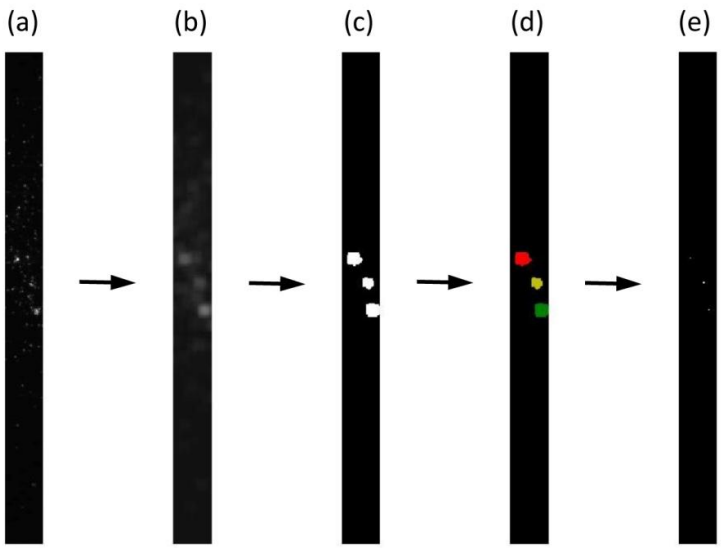

Figure S1. The frame processing pathway: (a) original frame, (b) Gaussian blur frame, (c) binary bitmap where above the threshold are white (1's) and below are black (0's), (d) enumeration of islands and elimination of unrealistically small or large islands, and (e) approximate locations recorded by the computer.

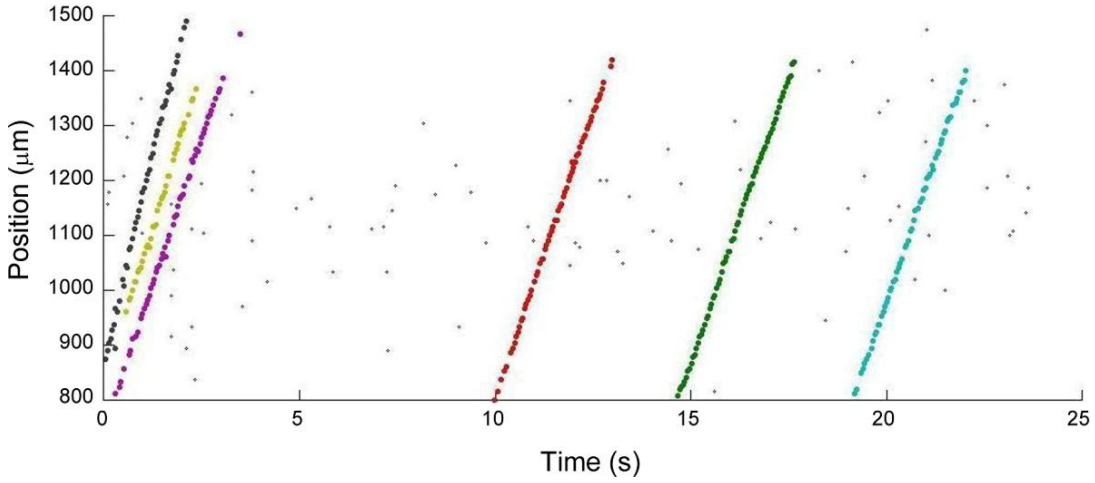

Figure S2. Position of FND-labeled cancer cells flowing through a microchannel as a function of time (or frame number). Cells are flowing from bottom to top. Dots that can be fitted into a straight line are grouped and considered as one cell signal. A total of 6 different cell signals are recorded here (within 25 s), each colored differently. Dots that cannot be fitted into straight lines are colored in gray and are regarded as random noises.

Figure S3. Photograph of the experimental arrangement for imaging FND-labeled cells in the blood vessels of a mouse ear.