

High Purity Microfluidic Sorting and Analysis of Circulating Tumor Cells: Towards Routine Mutations Detection

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Supplementary Information

Imaging procedure

A first screening step was performed using a 4x objective. A homemade MATLAB interface was developed to drive the microscope through Micromanager as an intermediate software. This interface allows for a fast and user-assisted recognition of the capture areas, followed by an automated high-speed scanning. Upon first implementation, a calibration of the system and camera alignment is performed. The first scanning step only uses the DAPI filter (excitation around 400 nm and emission around 465 nm) as a first nucleus recognition step. A dedicated high-speed focus algorithm (Matlab) was developed for that purpose. The obtained images were stitched using MATLAB to make a single, high-resolution image of the complete capture area. An automated cell counting is performed, and the exact position of each individual cell and corresponding column is stored in a database and made available for the user. This step takes about 5 minutes per capture zone (3 mm per 3 cm zones).

For further high-resolution analysis, the software uses each nucleus position as a reference in order to take multicolor, higher resolution images. For that purpose, x10 and x20 objectives are selected automatically and the stage is moved sequentially to the exact position of each nucleus using the x,y position database obtained in the first step. Before exposure, an automated z-focus is achieved. Due to the height of the microfluidic channel (around 50 μ m) cells can eventually be distributed all along the magnetic column direction. As a consequence, z-stacks (12 layers, 5 μ m between each) were taken for each column. A single image was finally reconstructed using maximum intensity projection in order to facilitate scan inspection by the user. All z-stacks were stored and remain available to the user in order to analyse ambiguous images, and extract the exact z position of each single cells on the column in case of cells superposition along the z axis.

Finally, the image of each cell can be extracted independently using an homemade morphology recognition algorithm. A database containing the x,y position of each cell and the associated images is generated. Cells are then stored for analysis such as staining intensity plots or manual visualization.

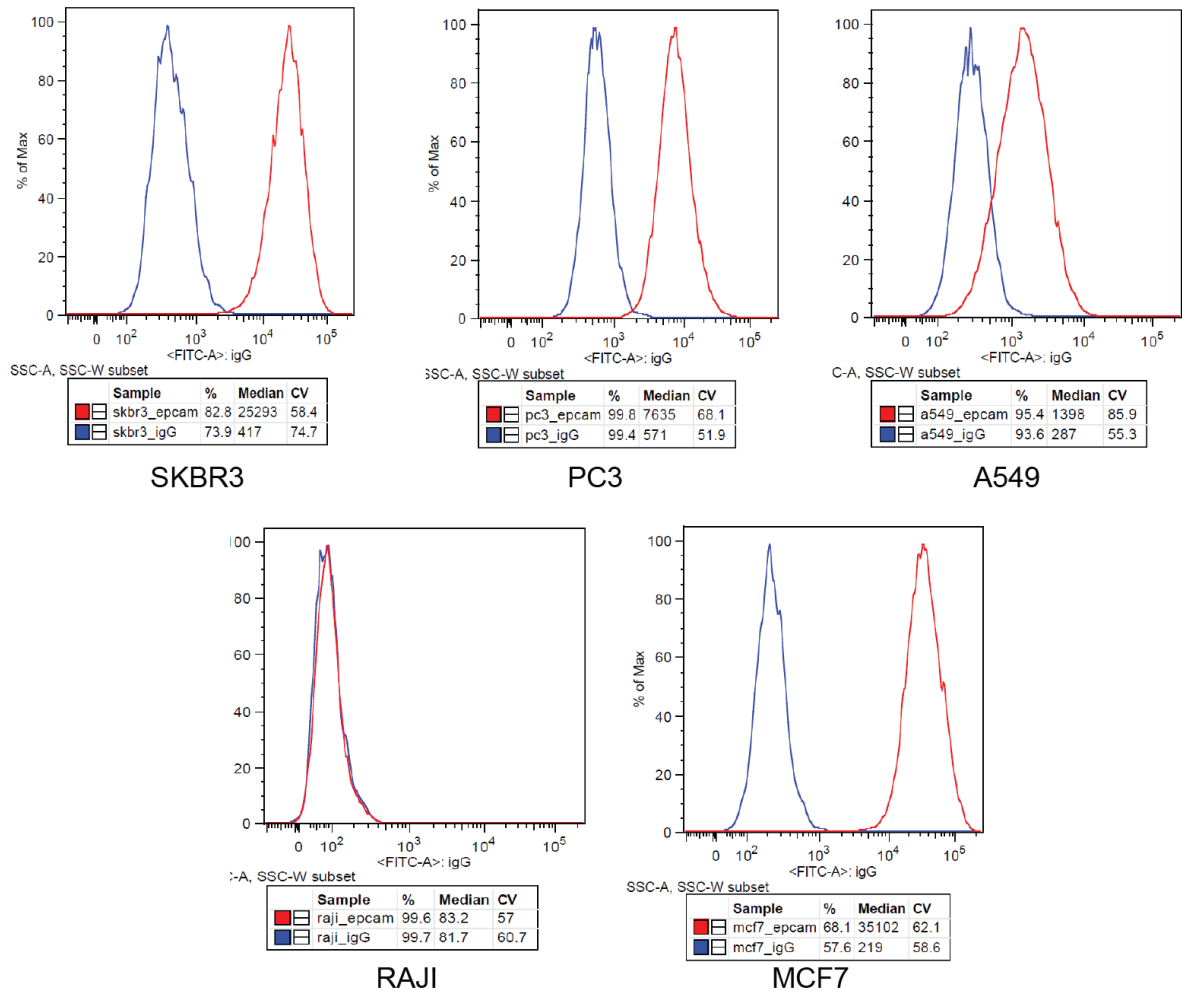


Figure S1: Flow-cytometry was used to measure EpCAM expression for each cell lines (MCF7, SKBR3, PC3, A549 and RAJI). EpCAM (red) and isotype control (igG, blue) are presented with median value and standard deviation.