

An automated multiplatform method for the efficient isolation of single rare cells

James Leary¹, Michael Zordan²

¹Basic Medical Sciences and Biomedical Engineering, Purdue University, Birck Nanotechnology Center, 1205 W. State, West Lafayette, IN, 47907, ²Biomedical Engineering, Purdue University, 206 S, Martin Jischke Drive, West Lafayette, IN, 47907

Background: The isolation of single rare cells is important to many fields of biology, notably in the studies of stem cell and cancer biology. The purification of rare cells by flow cytometry based cell sorting is difficult because there is always a trade-off between yield and purity. When there are only a small number of rare cells in the initial sample, yield and purity are both necessary. Flow cytometry based cell sorting is also not very amenable to the processing of a small number of cells. The Laser Enabled Analysis and Processing (LEAP)TM instrument (Cytellect, Inc.) is an adherent cell cytometer that performs fluorescence imaging and has the ability to sort cells by laser ablation. The LEAPTM system processes cells in the wells of a multiwell plate so even a single cell can be processed. Therefore the strengths of traditional flow cytometry based cell sorting and LEAPTM purification can be combined to efficiently isolate single rare cells.

Methods: Peripheral blood was spiked with a rare fraction of A548 lung carcinoma cells. A first pass enrichment sort was conducted using an iCyt Reflection high speed cell sorter that is equipped with a microwell plate holder collection stage. The LEAPTM instrument will identify the rare cells using automated image analysis and eliminate any contaminating cells from the first pass sort by laser ablation. For single cell cloning studies, the LEAPTM instrument can be programmed to leave only a single live rare cell in each well.

Results: We have successfully sorted rare cell fractions present at frequencies lower than 1 in 10⁵ total cells directly into 384 well plates. These cells were then purified to a single clone in each well at greater than 90% efficiency. These cells are vital and grow clonally with similar doubling times in comparison to normal culture and ultimate limiting dilution clonal growth assays.

Conclusions: Rare cell fractions can be efficiently purified with a high yield using a flow based enrichment sort followed by LEAPTM based purification. The technique should be capable of isolating rare cells at frequencies far below 10⁻⁶. This technique can successfully purify a sample down to a single rare cell. This technique can purify live cells for subsequent vital assays, or single cell biochemical assays. Using both traditional flow based cell cytometry and LEAPTM purification combines the strengths of both techniques, while each technology will mitigate the shortcomings of the other.