

BME 695N

Engineering Nanomedical Systems

Lecture 6

Rare-event targeting of cells in-vitro and in-vivo

James F. Leary, Ph.D.

SVM Endowed Professor of Nanomedicine
Professor of Basic Medical Sciences and
Biomedical Engineering

Member: Purdue Cancer Center; Oncological Sciences Center;
Bindley Biosciences Center; Birck Nanotechnology Center

Email: jfleary@purdue.edu

I. Assessing nanomedical system (NMS) targeting at the single cell level

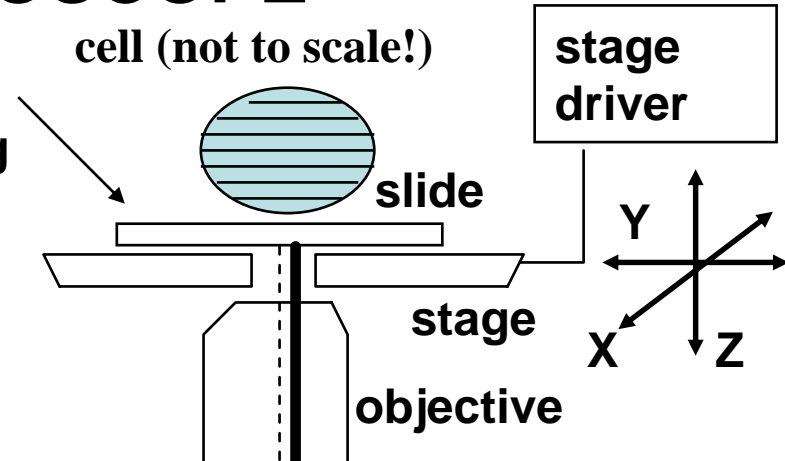
- A. Fluorescent labeling of NMSs
- B. First estimates of NMS binding by fluorescence microscopy
- C. Internal or external binding by confocal microscopy
- D. Single-cell image/confocal analysis
- E. Flow cytometric quantitation of NMS binding to specific cell types

II. Image confocal analysis of NMS binding to single cells

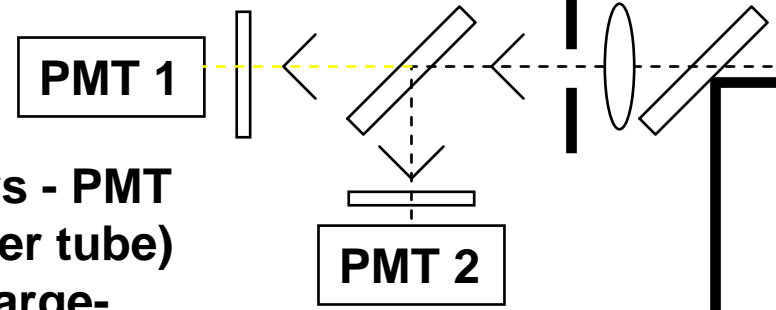
- A. Ability to scan/locate cells of interest
- B. Photobleaching challenges
- C. Optical sectioning for 3D location of NMSs on/within cells

A GENERALIZED IMAGE CYTOMETER/ CONFOCAL MICROSCOPE

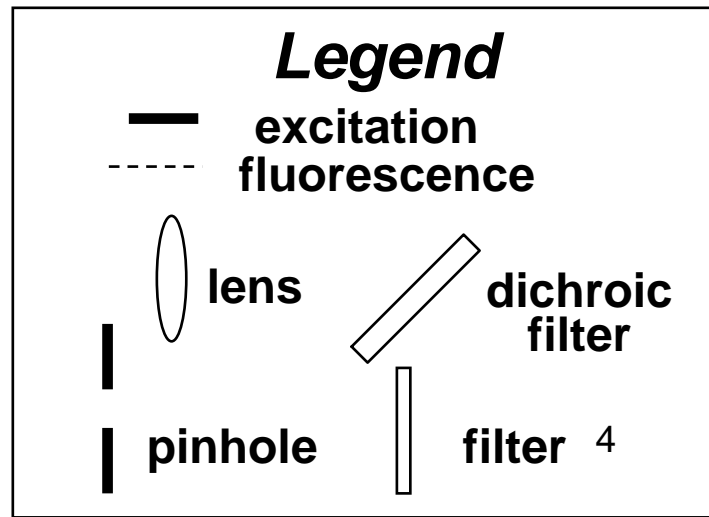
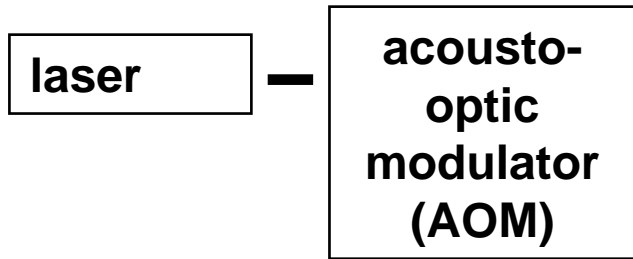
single-cell micro-manipulation by (1)
micromanipulators or (2) optical trapping



fluorescence
filter assembly



photodetectors - PMT
(photomultiplier tube)
or CCD (charge-
coupled device)

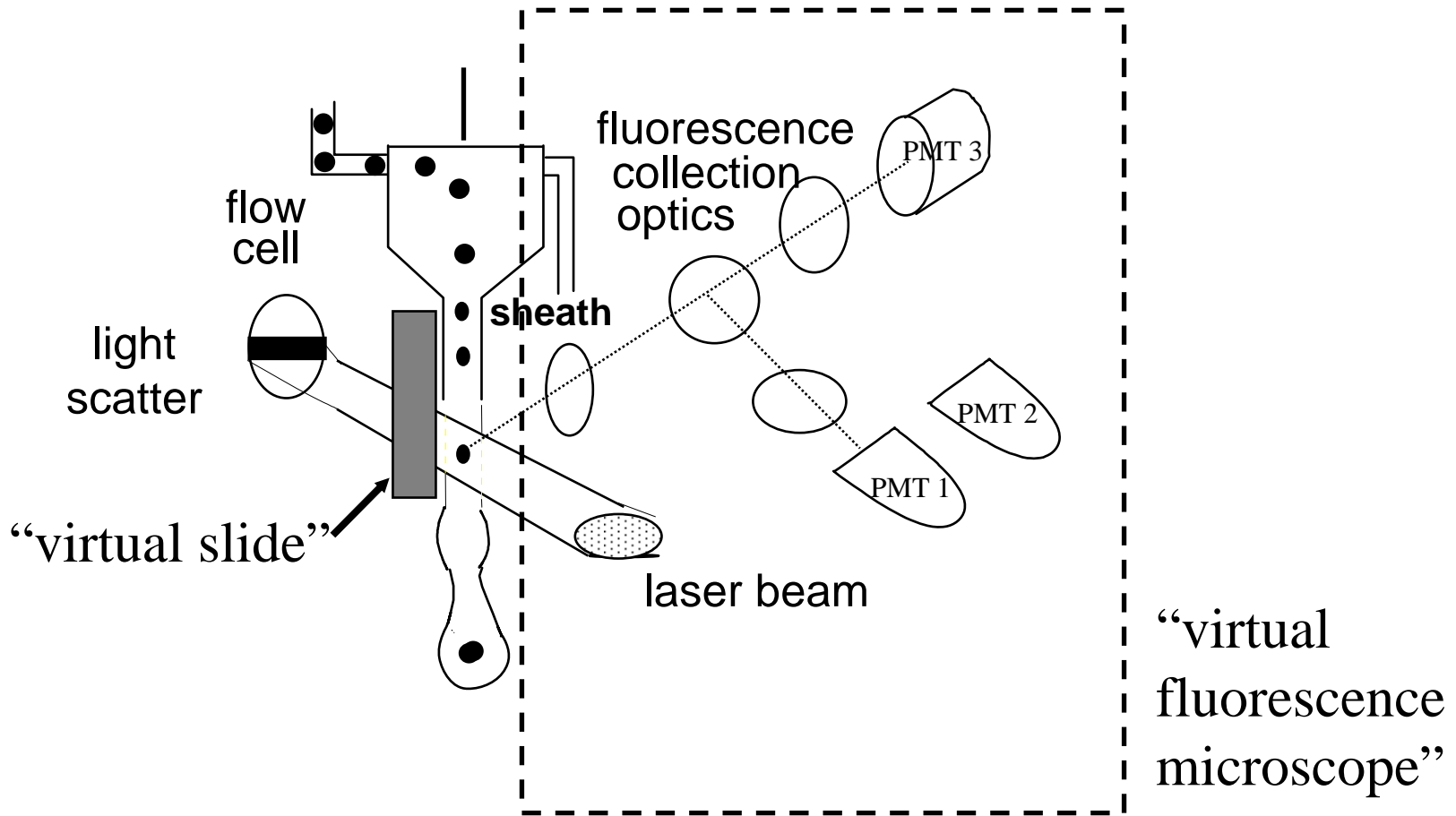


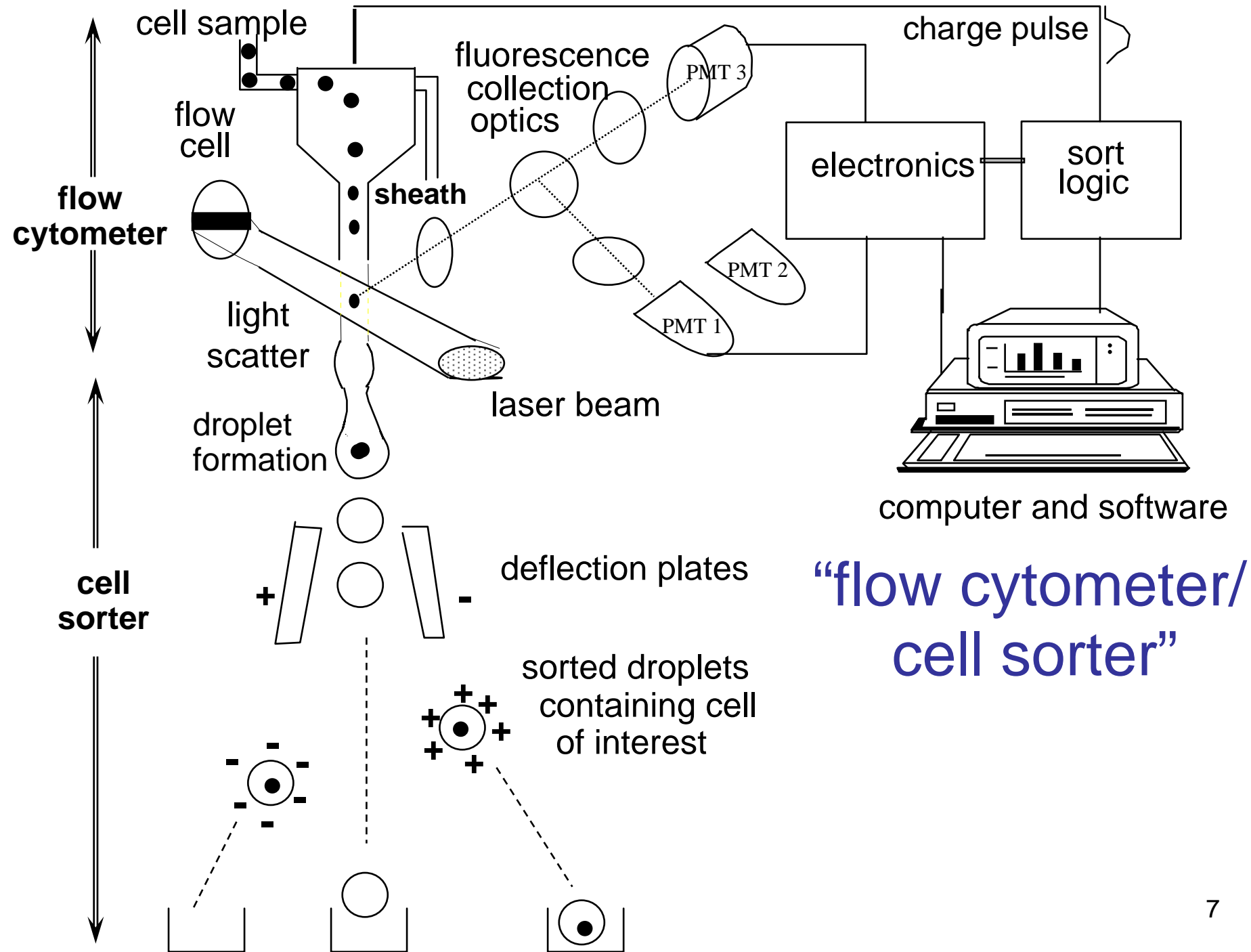
III. A quick overview of flow cytometry for quantitative multiparameter analysis of single cells

- A. Basic principles
- B. Capabilities and limitations
- C. Use for assessing specificity and sensitivity

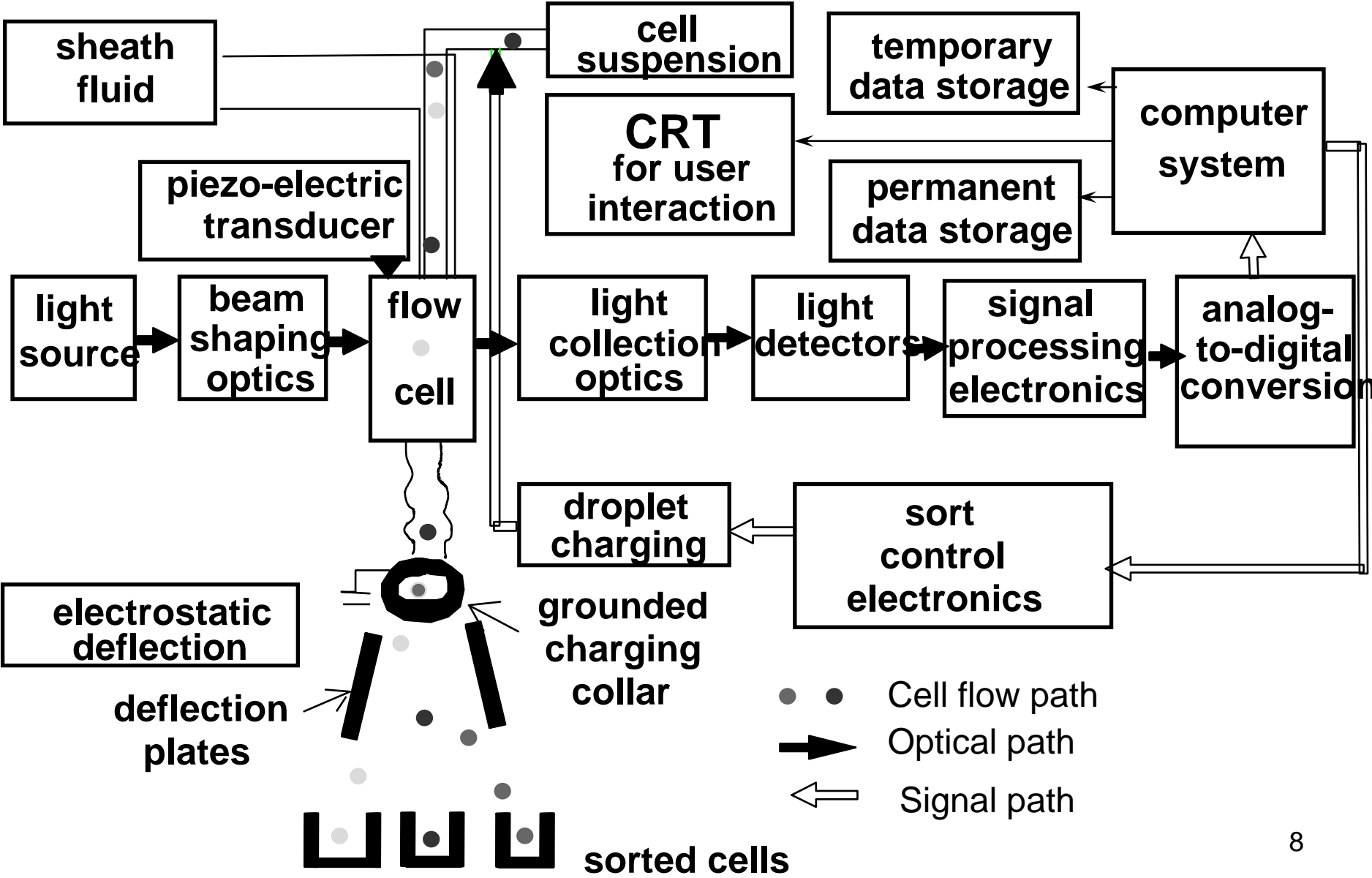
Flow Cytometer – Just another form of a fluorescence microscope!

(just tip the picture (not the flow cytometer!) on its side)

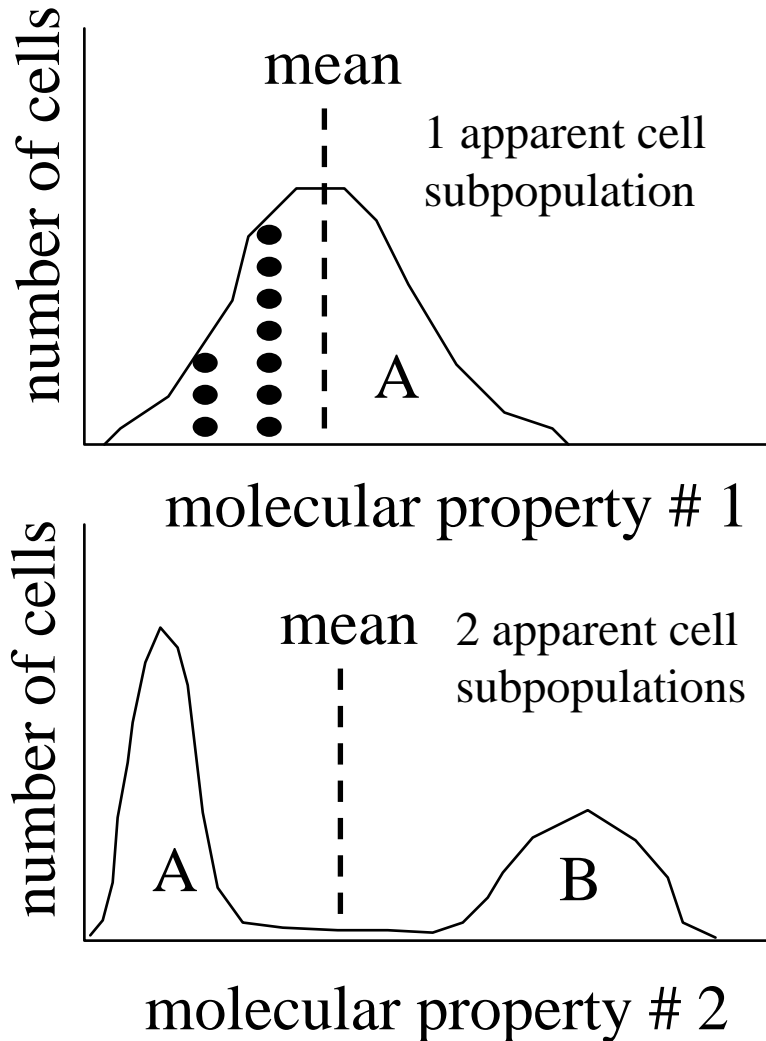




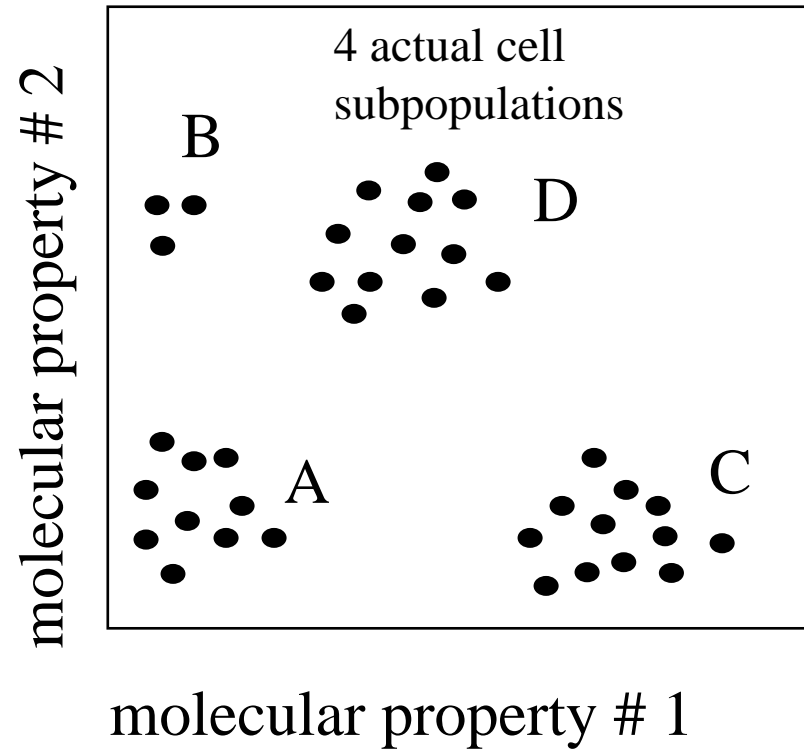
GENERALIZED FLOW CYTOMETER/CELL SORTER



Importance of Correlated, Multiparameter Quantitative Single Cell Molecular Measurements



quantitation and correlation of two or more molecular properties



IV. Rare-event analysis of NMS targeting to desired cells

- A. Basic concepts of rare-event analysis
- B. Strategies for rare cell detection
- C. More advanced flow cytometry for ultra-rare cell detection
- D. Examples of rare cell detection
- E. Rare cell sampling statistics

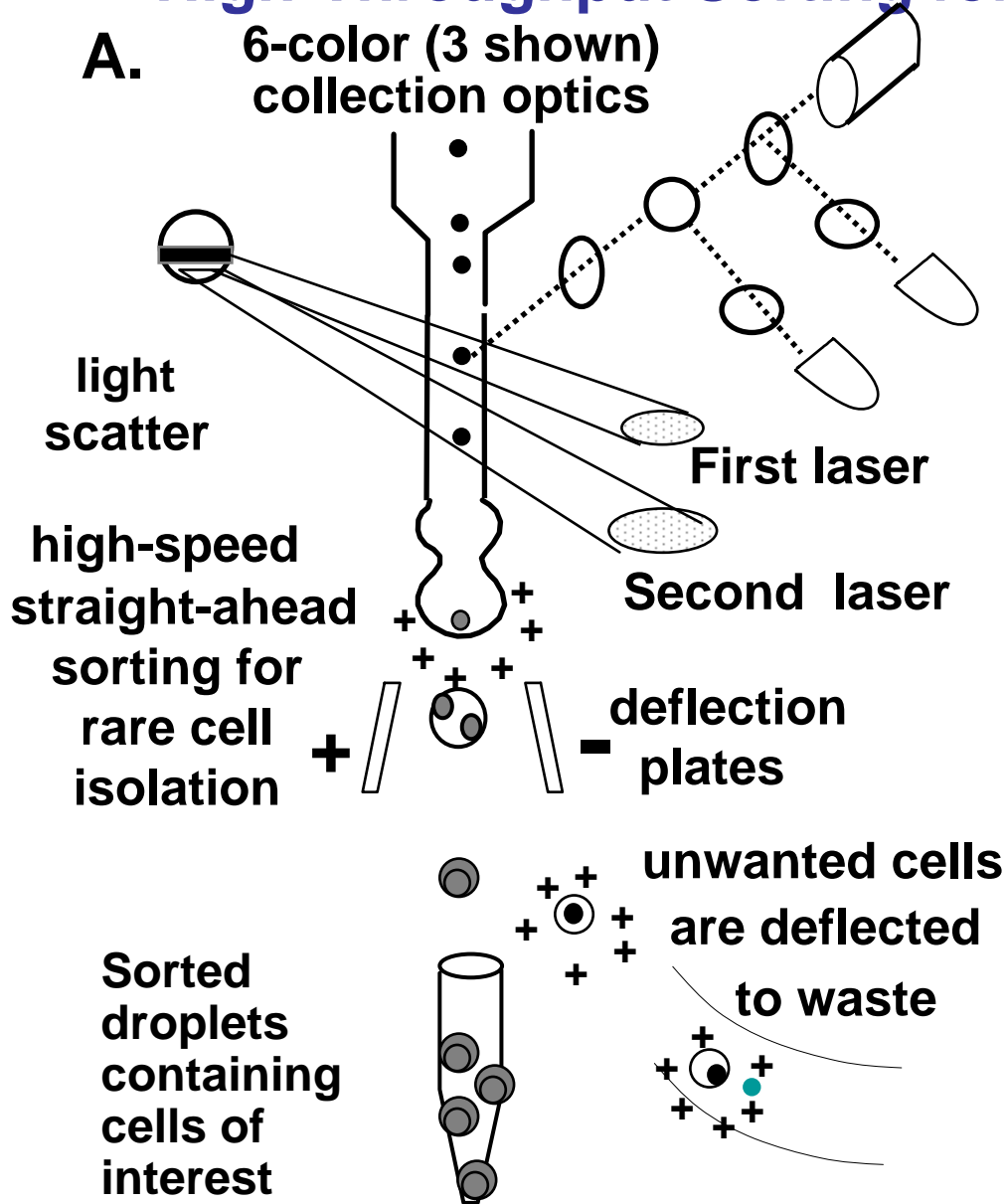
High-speed flow cytometry/cell sorting



MCU



Advanced High-Speed Flow Cytometer / Cell Sorter for High-Throughput Sorting for Functional Genomics



SPECIAL FEATURES

B. High-speed (>100 000 cells/sec) real-time data classification and error-checking

C. Generation of multi-variate classifier functions of input parameters in real-time

D. Real-time, second-stage data classification & cell sorting

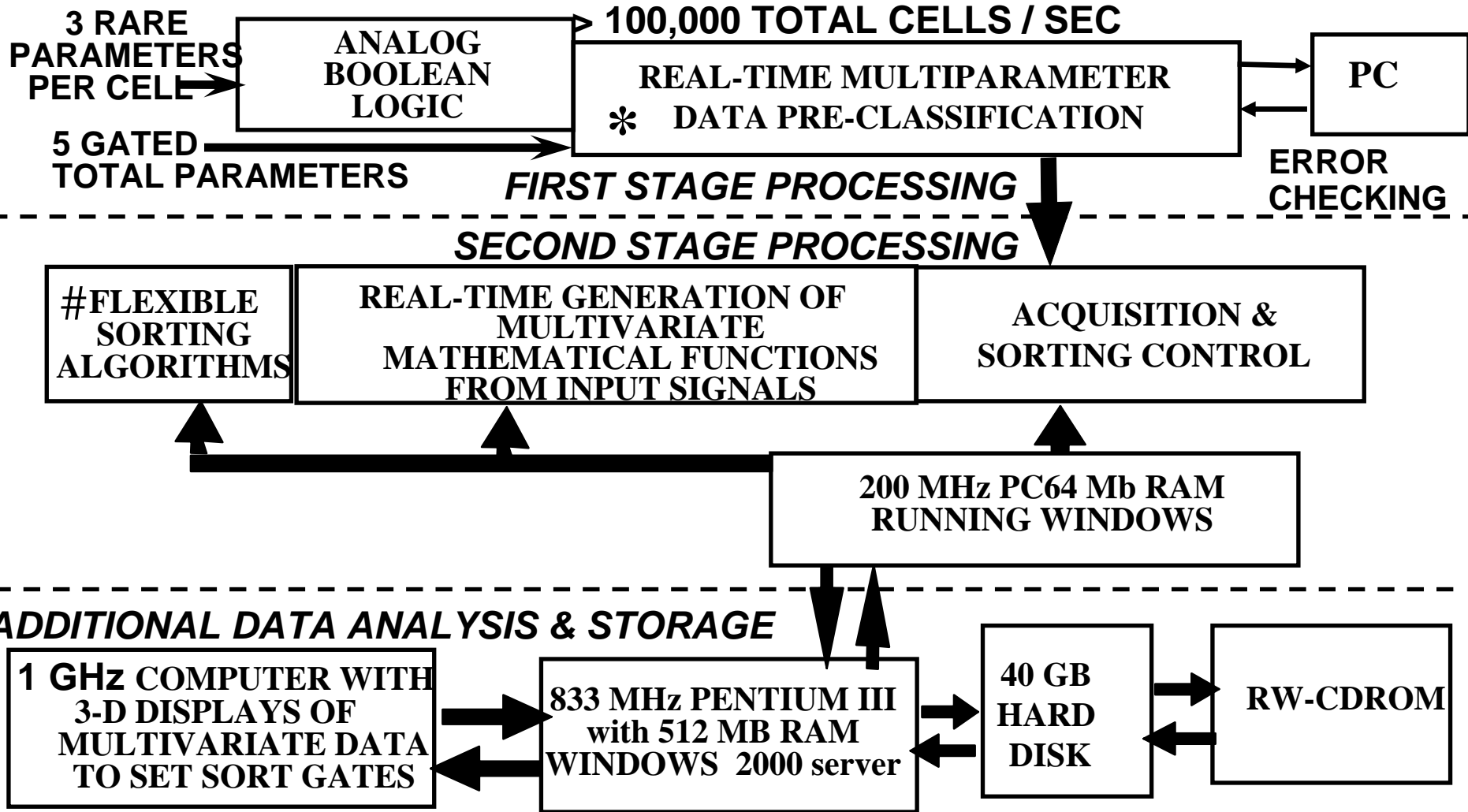
E. Algorithmic "flexible sorting" strategies for improved yield and purity of sorted cells

F. High-resolution cell sorting with costs of sorting misclassification

G. Single-cell sorting for subsequent PCR characterizations

FOR FUNCTIONAL GENOMICS

Schematic of High-Speed Flow Cytometer/Cell Sorter with Sophisticated Multiparameter High-Throughput Cell Separation for Functional Genomics



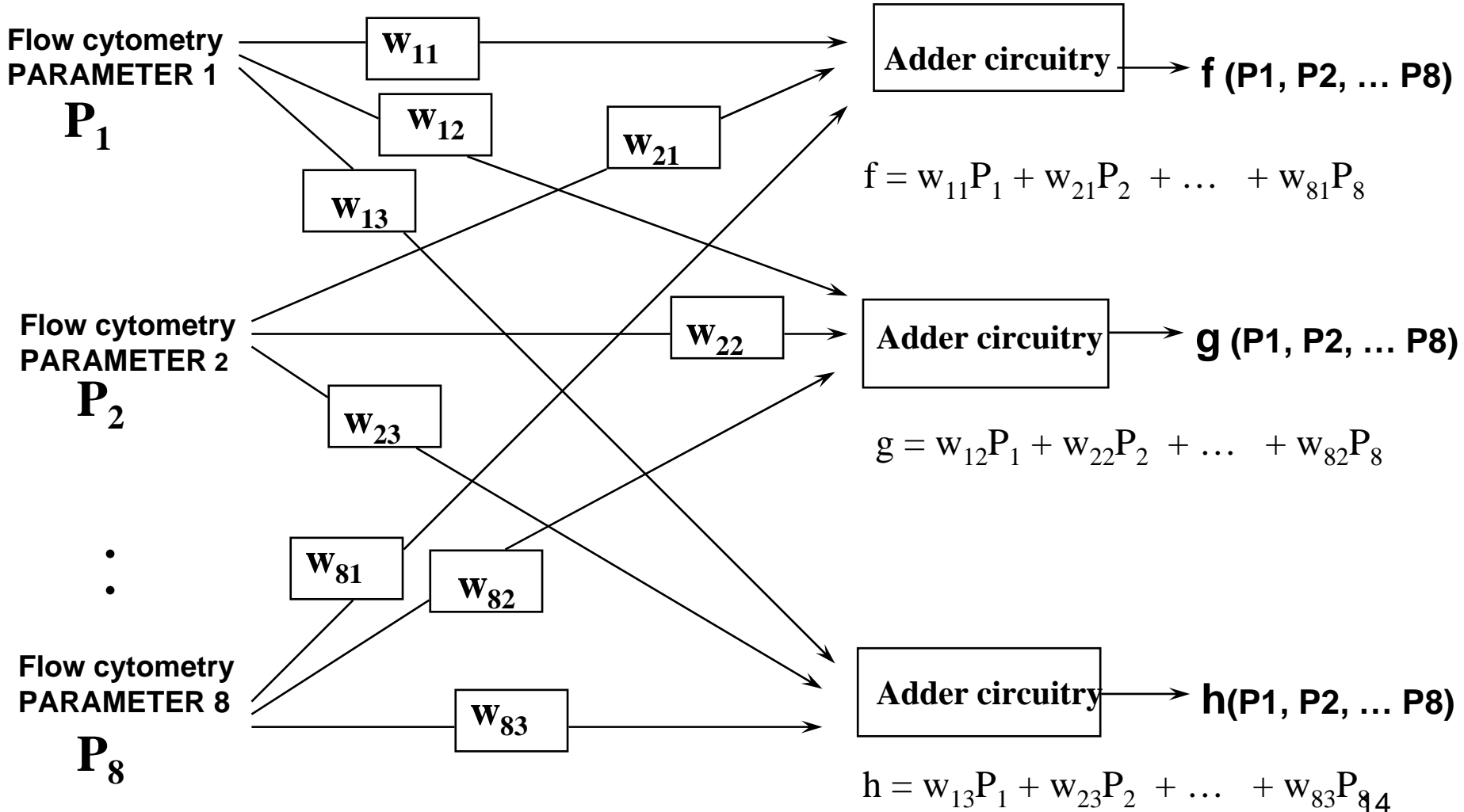
* U.S. Patent 5,204,884 (1993)
and 5,804,143 (1998)

U.S. Patents 5,199,576 (1993)
and 5,550,058 (1996)

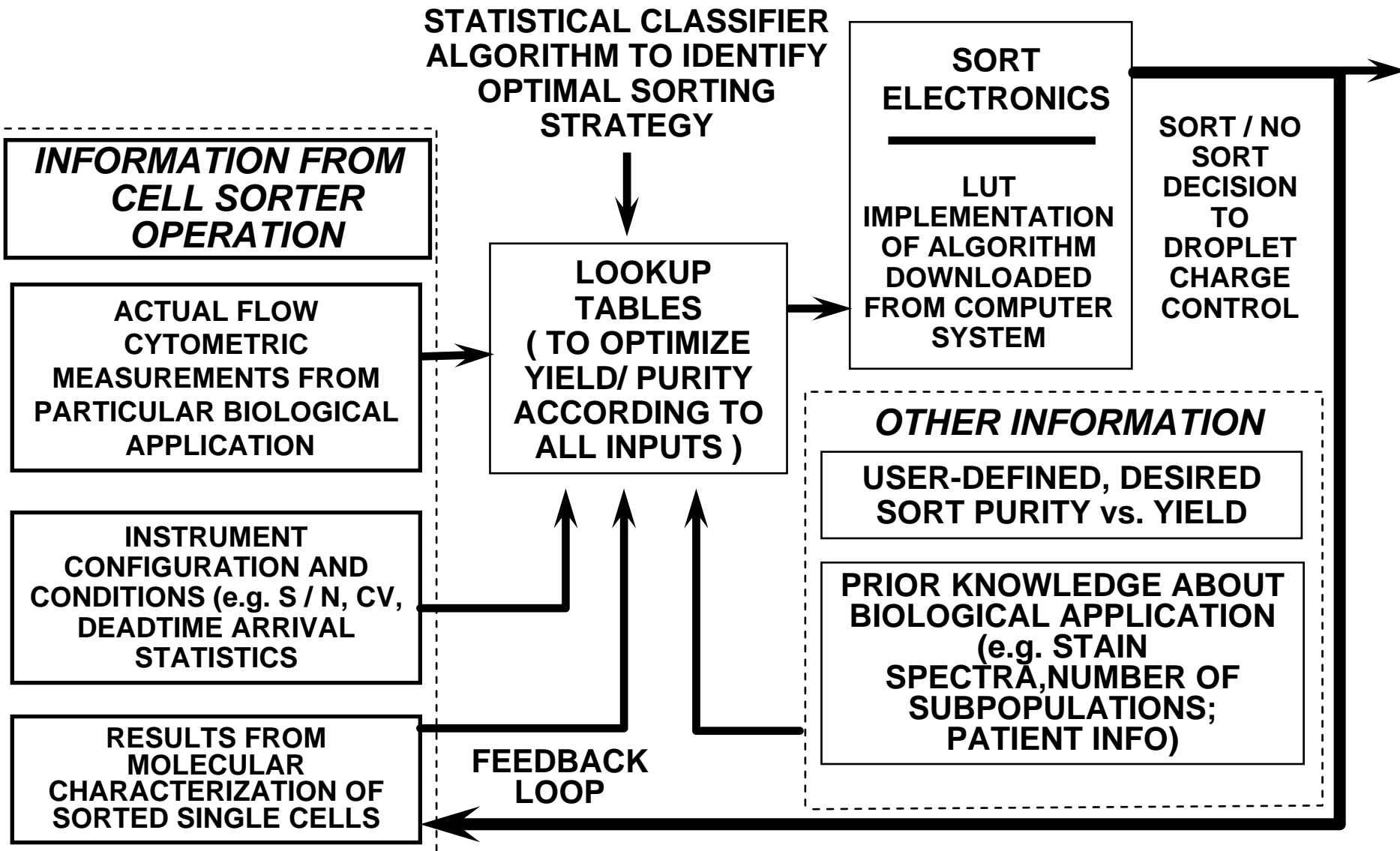
Real-time formation of multivariate classifier functions

LUTs for real-time generation of weighting factors (w_{ab}) or a transfer function

multivariate classifier functions



Sort Yield/Purity Optimization Using Embedded Algorithm for Real-time Consideration of Penalties of Misclassification



Rare Cell Frequency is Correct!

Rare Cell Sampling Statistics – How many cells do you need to sample to be sure (with 95 percent confidence) that your rare cell frequency is correct?

Table 1		Frequency of Cells with Selected Characteristics = 10 E-06 (0.95 level of assurance)					
Desired number of cells with Selected Properties	Total number of sort decisions	Time (seconds) required to collect desired cells					
		Sorting Rate (Sort Decisions/Sec)					
		2500	5000	10000	20000	50000	100000
1	2,990,000	1196	598	299	150	60	30
10	15,705,214	6282	3141	1570	785	314	157
100	116,997,126	46799	23399	11700	5850	2340	1170
1000	1,052,577,091	421031	210515	105258	52629	21052	10526

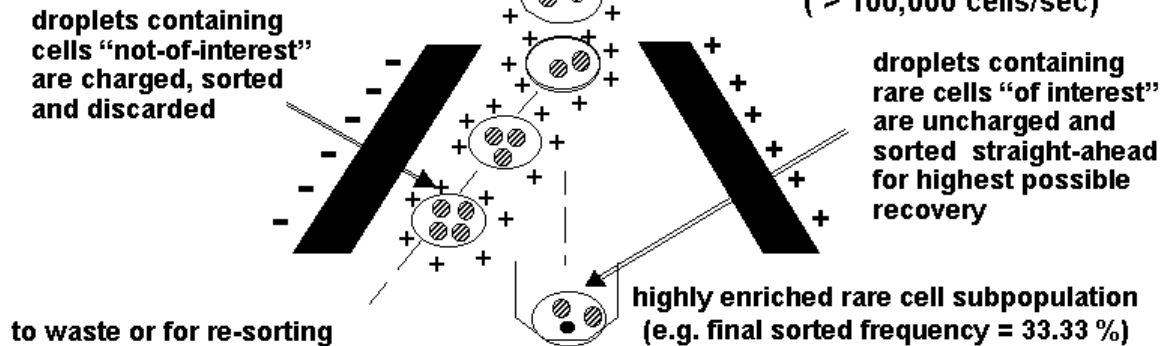
Source: Rosenblatt et al., 1997.

Concept of high-throughput “enrichment sorting”

Principle: The average number (the exact number can be predicted by queuing theory and Poisson statistics) of cells per sorting unit is approximately equal to the number of total cells/sec divided by the number of sorting units/sec

- non-rare cell, “not -of-interest”
(e.g. original frequency = 99.999 %)
- rare cell, “of interest”
(e.g. original frequency = 0.001 %)

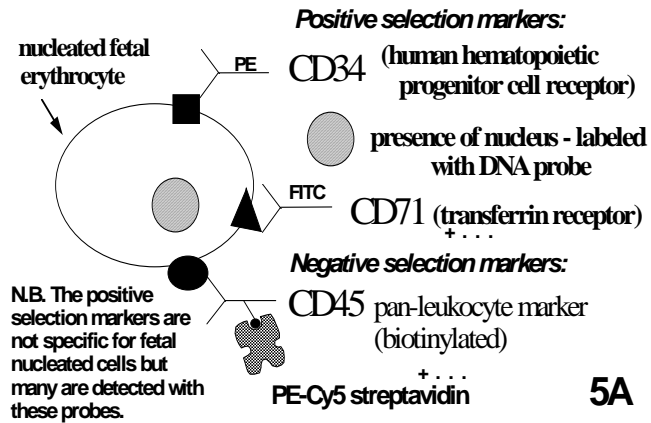
Example: high-speed sorting
(> 100,000 cells/sec)



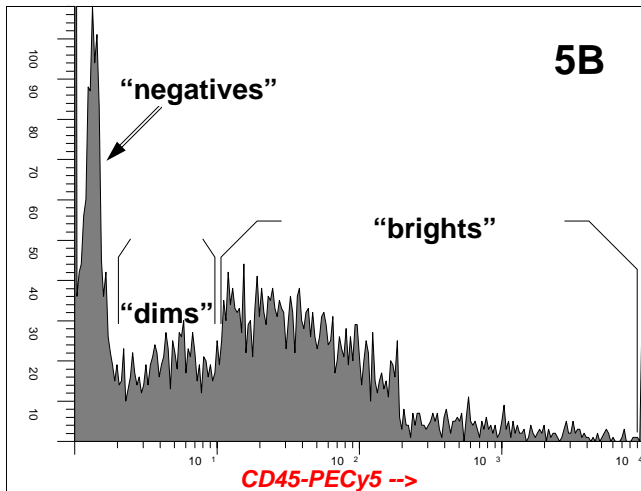
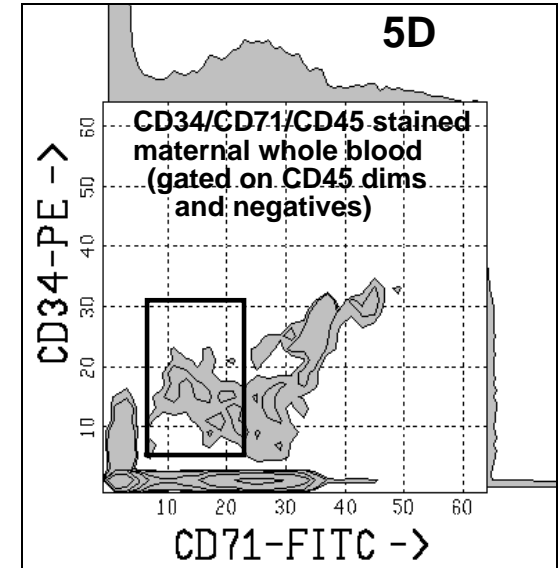
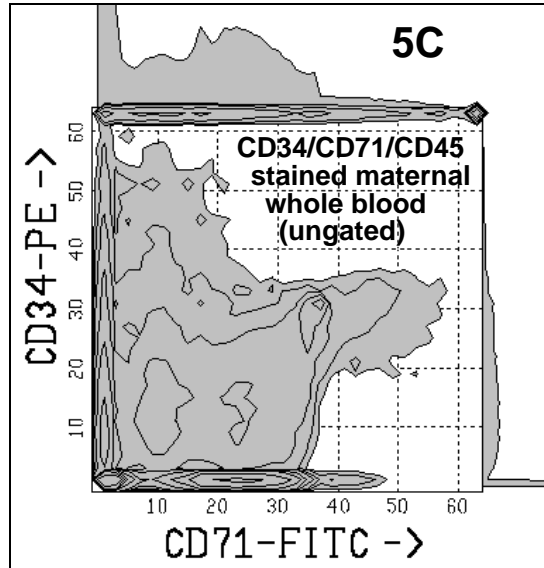
Cell sorters are much more efficient at very high throughput speeds in terms of the enrichment factor from the original rare cell concentration to the final sorted rare cell concentration. In this concept, a cell of original frequency of 10^{-5} (1 rare cell per 100,000 total cells) can be sorted at 100,000 cells/s with a sorter capable of generating 33,000 droplets/s. A high-speed first-pass sort enrichment of more than 30,000-fold, based on up to three rare parameters and five additional total parameters, can be attained. Sorted cells can then be resorted to any desired purity based on the quality of the selection probes.

Biomarker strategies for labeling, detecting and isolating ultra-rare cells

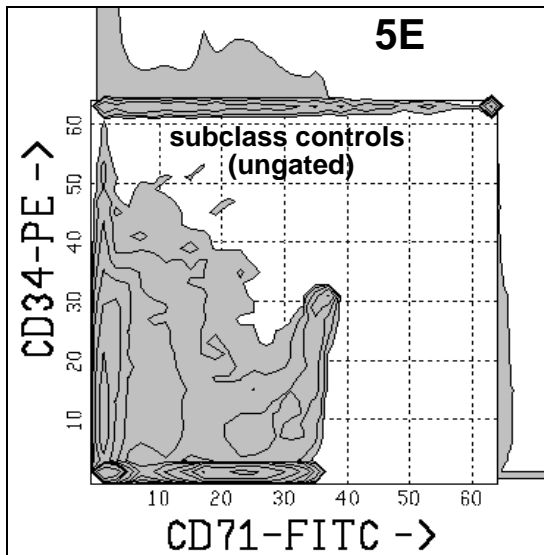
LABELING STRATEGIES FOR DETECTION OF NUCLEATED FETAL ERYTHROCYTES IN MATERNAL BLOOD



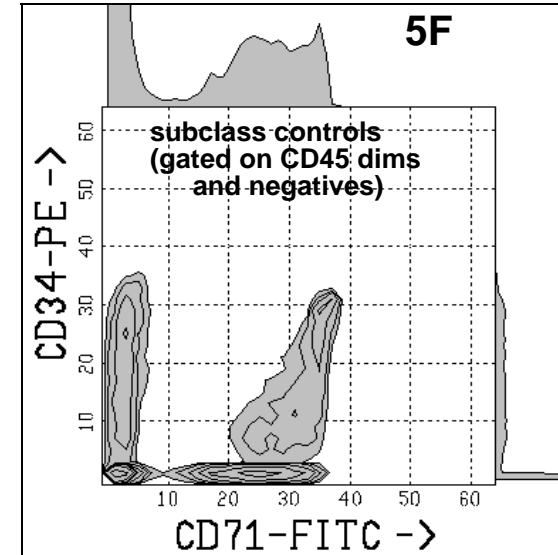
5A



5B



5E



5F

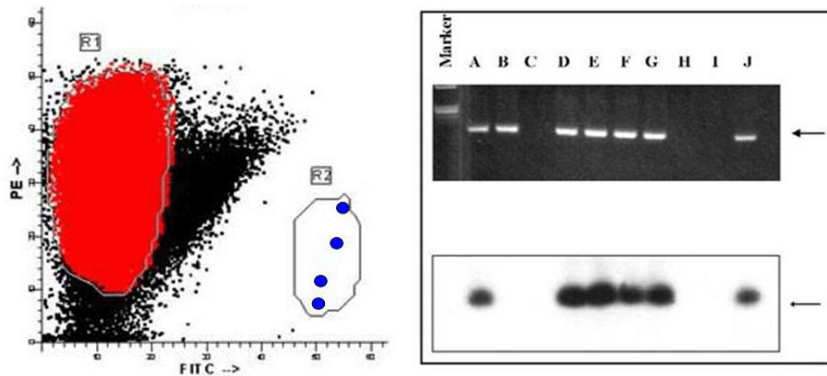
Diagnosics/Therapeutics

Example of a Unique Technology: High-Throughput Technologies for Drug Discovery and Cancer Diagnostics/Therapeutics

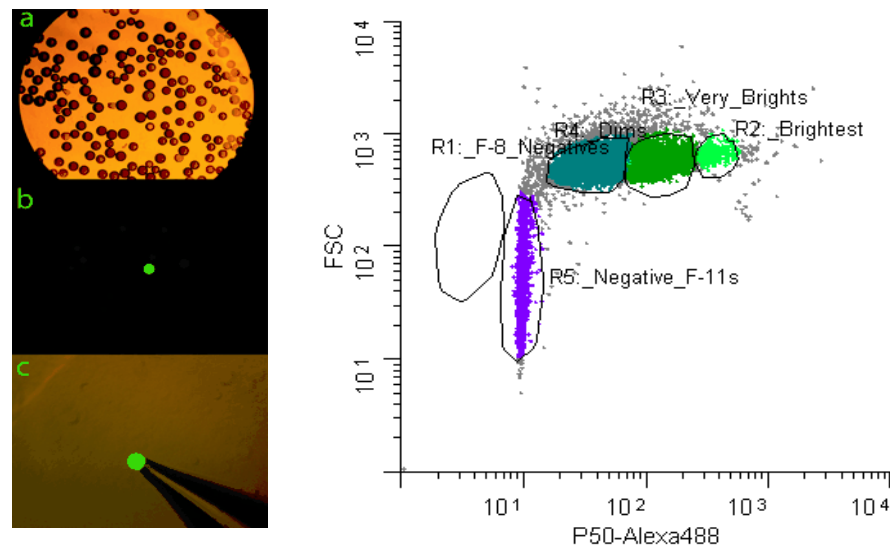


This high-speed flow cytometer/cell sorter is the world's fastest instrument and is used for separating rare cells or particles of interest.

New technologies for the detection and isolation of very rare tumor cells in cancer patients

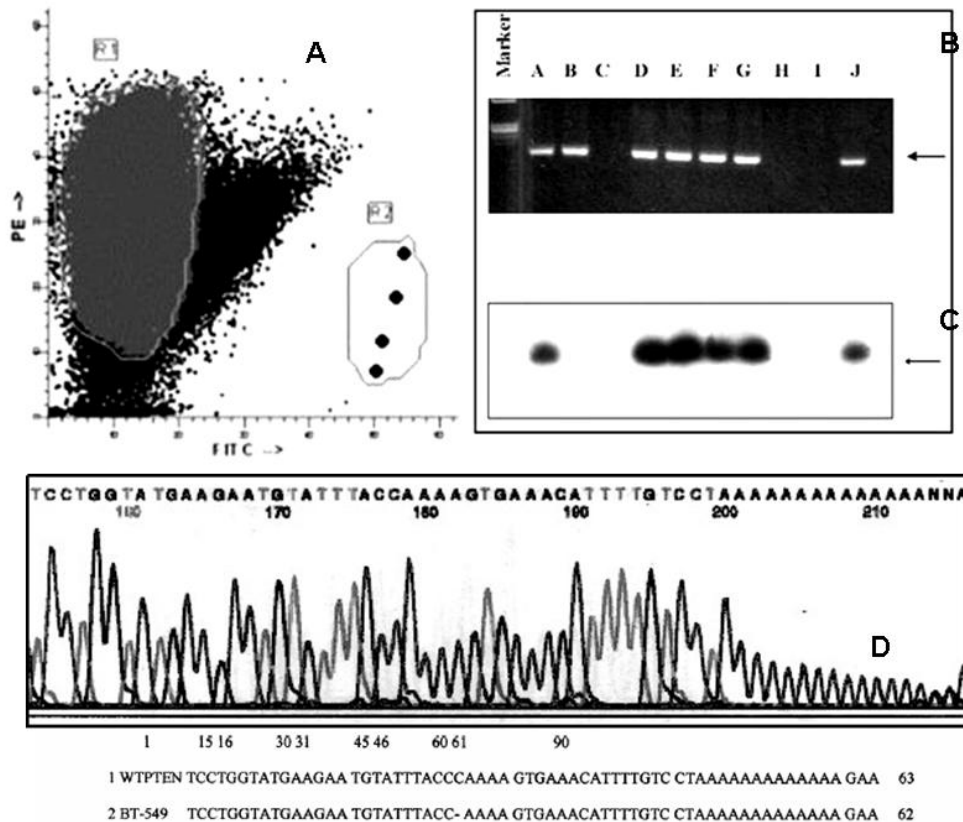


Early cancer detection and monitoring of patients in remission requires very high-speed detection, isolation and molecular characterization technologies for rare single tumor cells (●).



Sorting of thioaptamer combinatorial chemistry library beads with bound protein, is one way to isolate a specific drug. Up to 100 million drug candidates can be screened in a single day using high-throughput technologies.

High-throughput (>100,000 cells/sec) cell and bead-based chemistry can be used to explore vast cell and bead libraries to search for the cells or beads with optimal characteristics. Isolated rare cells or beads can be subsequently analyzed or sequenced. We are using these methods to select nanoparticle targeting molecules for nanomedicine.



Isolation and molecular characterization of ultra-rare breast cancer tumor cells from human blood

Figure 11: Flow cytometric results from a defined cell mixture of 10^{-5} frequency MCF-7 cells in a major population of human CEM/C7 T cells. Cells were labeled with a phycoerythrin (PE)-conjugated anti-CD45 antibody and a fluorescein isothiocyanate (FITC)-conjugated anti-cytokeratin antibody. A small subpopulation of rare MCF-7 cells was detected in region R2 in an aliquot of the sample. Cells in this region were then sorted at the single-cell level for subsequent PCR analysis, TA cloning, and DNA sequencing. The four tumor cells, shown in this aliquot of cell sample, have been highlighted as dark enlarged circles in the flow cytometric distribution for easier viewing. The right-hand panel shows ECL detection of PCR-amplified sequences from sorted, rare, single tumor cells as shown in left-hand panel. The top panel showed that nested PCR product on 2% agarose gel stained with ethidium bromide. The lower panel showed the result of Southern blotting with HEA DQalpha type 4 probe. The result indicated that the sorting efficiency is 7 of 10, and the southern bolt showed 6 of 10 is MCF-7 cells (lanes A, D, E, F, G, and J). The lower subpanel shows the result of ECL Southern blotting with HLA DQa type 4 probe. The result indicated that the overall sorting efficiency was seven recovered single rare cells out of 10 (a fairly typical recovery over many experiments), and the Southern blot revealed that six of those seven sorted cells were MCF-7 cells (lanes A, D, E, F, G, and J), showing that the sort classification was fairly accurate. DNA sequencing of the PTEN gene region from a single sorted cell (top) and alignment (bottom). The alignment shows that the PTEN mutation consists of a missing single base pair at nucleotide 61 of exon 8. These results show successful detection of a single base-pair mutation in a single sorted cell.

In Development: An Ultra-fast ($> 10^6$ cells/sec) parallel/exponential microfluidic cell sorter

(19) **United States**

(12) **Patent Application Publication**
Leary et al.

(10) **Pub. No.: US 2003/0153085 A1**
(43) **Pub. Date: Aug. 14, 2003**

(54) **FLOW SORTING SYSTEM AND METHODS REGARDING SAME**

(75) **Inventors: James F. Leary, Galveston, TX (US);
Christopher J. Frederickson,
Galveston, TX (US)**

Correspondence Address:
**MUETING, RAASCH & GEBHARDT, P.A.
P.O. BOX 581415
MINNEAPOLIS, MN 55458 (US)**

(73) **Assignee: NeuroBioTex, Galveston, TX (US)**

(21) **Appl. No.: 10/340,520**

(22) **Filed: Jan. 10, 2003**

Related U.S. Application Data

(60) **Provisional application No. 60/347,620, filed on Jan. 10, 2002.**

Publication Classification

(51) **Int. Cl.⁷ G01N 33/48**
(52) **U.S. Cl. 436/63; 422/73; 210/695;
210/222; 436/1**

(57) **ABSTRACT**

A system and method for sorting of objects that includes a pathway network that has a plurality of pathways and one or more branch points. A fluid composition including one or more objects can be transported through the pathway network, where one or more of the objects are analyzed and sorted at one or more branch points based on the analysis of the objects.

References

1. Leary, J.F.: "Strategies for Rare Cell Detection and Isolation" In: Methods in Cell Biology: Flow Cytometry (Edited by Z. Darzynkiewicz, J.P. Robinson, H.A. Crissman), vol. 42: pp. 331-358, 1994.
2. Leary, J.F. "Ultra High Speed Cell Sorting" *Cytometry Part A* 67A:76–85 (2005)
3. Rosenblatt, J.A., Hokanson, J.A., McLaughlin, S.R., Leary, J.F.: A Theoretical Basis for Sampling Statistics Appropriate for the Detection and Isolation of Rare Cells Using Flow Cytometry and Cell Sorting *Cytometry* 26: 1-6; 1997.