Flow Cytometry for Food Microbiology

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Single cell (particle) based analysis method for:

- Monitoring Fermentation Processes
- Rapid Pathogen Detection, Enumeration, Identification
- ...

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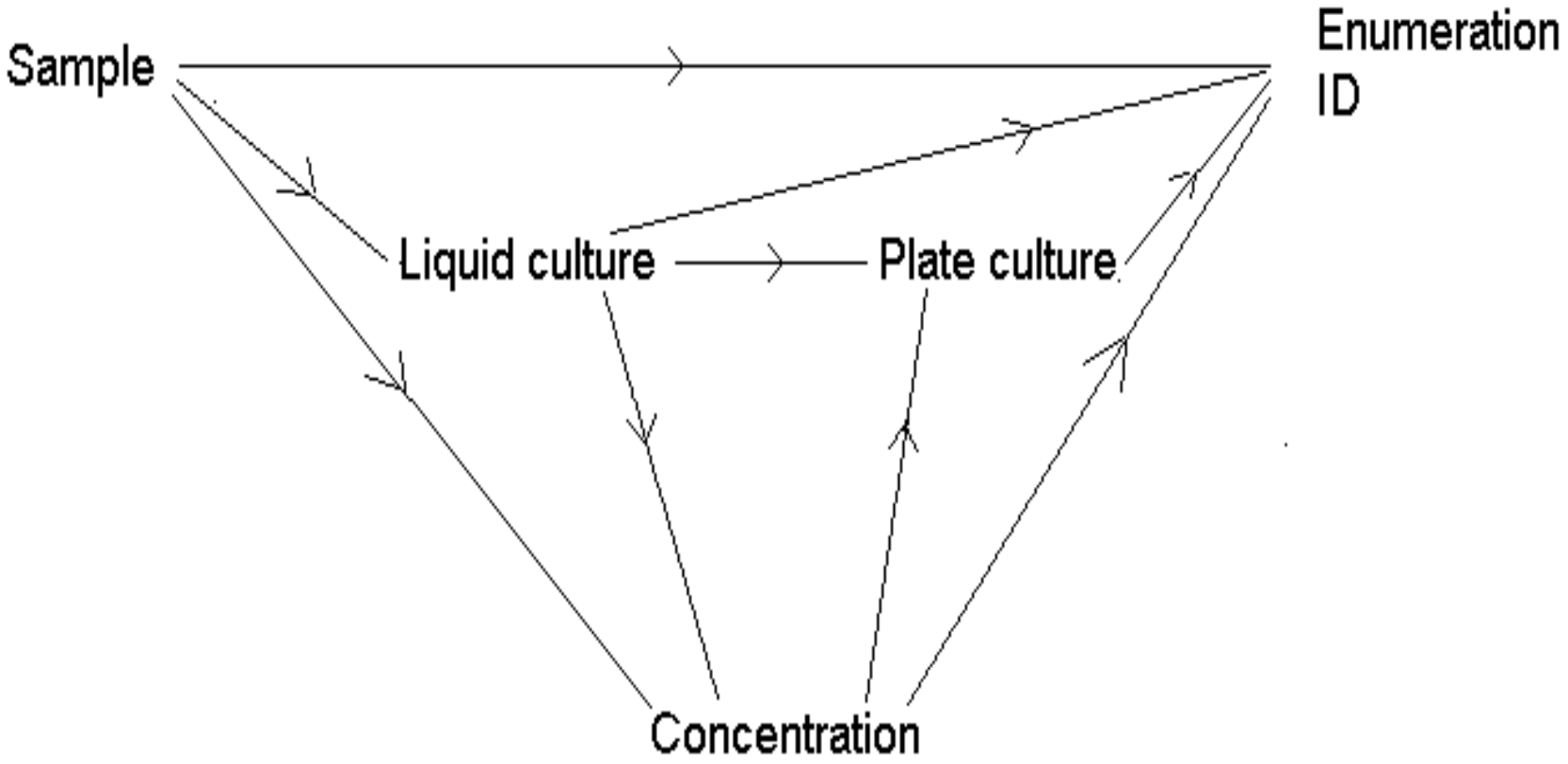
Microbial Detection & Identification



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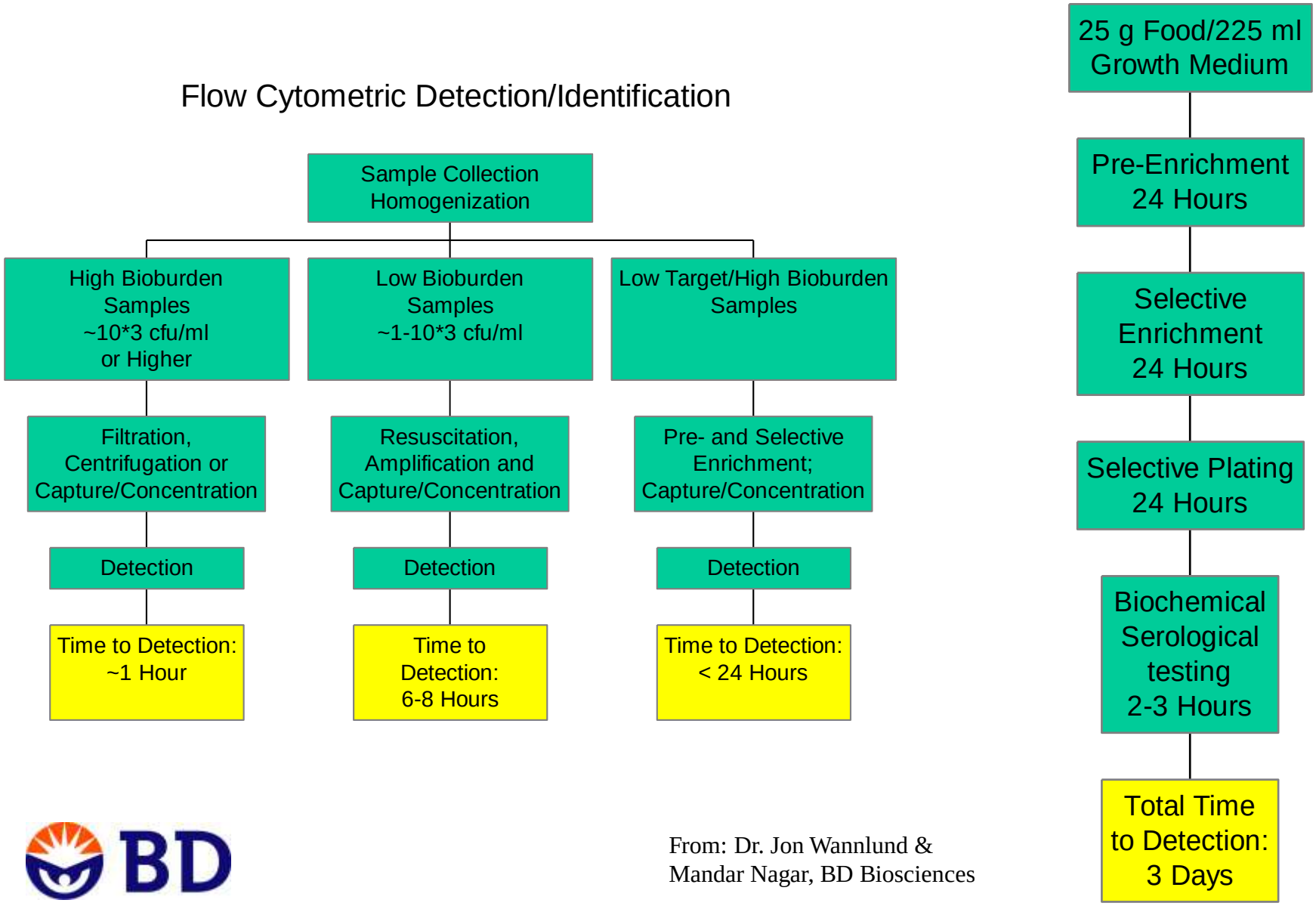
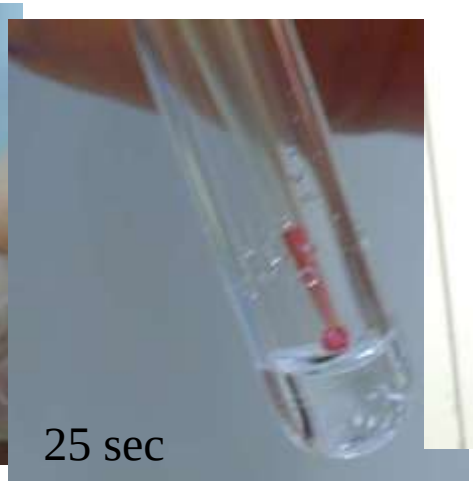
Workflow for Food Pathogen Analysis

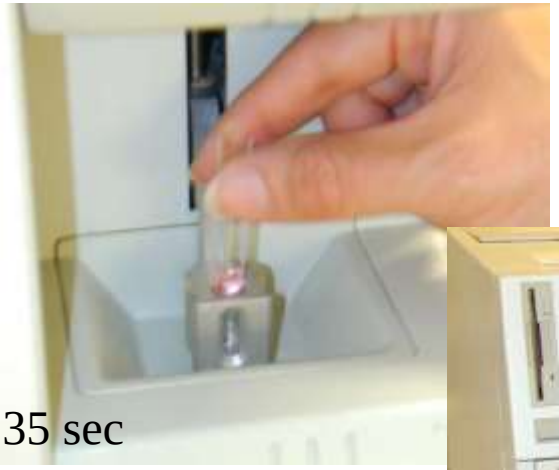


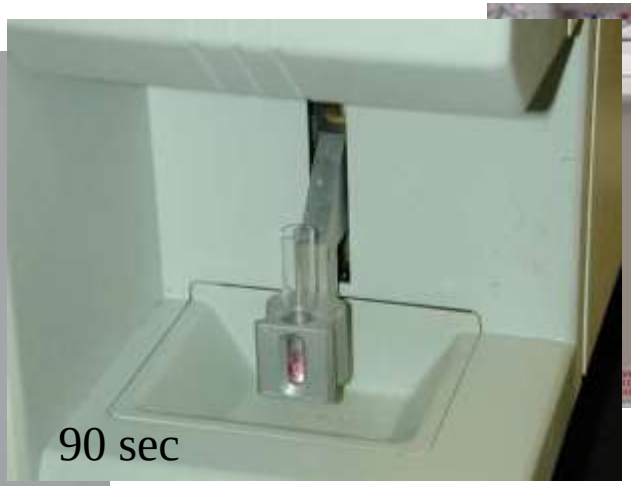
From: Dr. Jon Wannlund &
Mandar Nagar, BD Biosciences

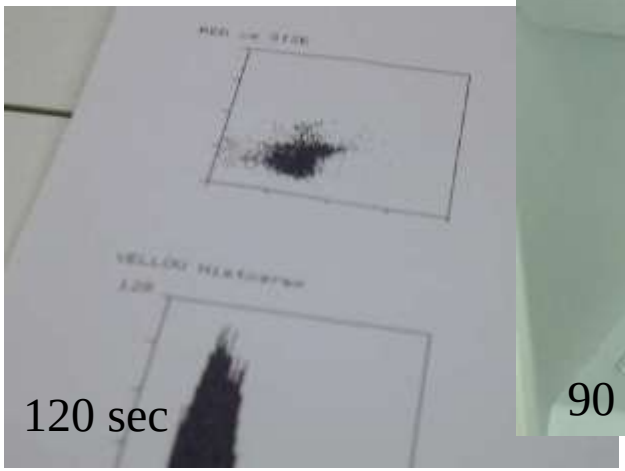
Flow Cytometry for Microbiology

Assay sequence from sample to result









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Rana Alsharif, BD Biosciences

Flow Cytometry for Microbiology

Expert Opinions

"4.1 Flow cytometry
... The sensitivity of the technique is very high:
as few as 10^2 yeast cells and about 10^2 - 10^3 bacterial cell per ml can be detected, with results being obtained within a few minutes. Because of its high sensitivity, flow cytometry is very suitable for detecting low numbers of specific organisms in fluid or rinses. ..."
E. de Boer et al(1999) Int J Food Microbiol 50:119-130

"**Prospects**
Flow cytometry is coming of age as a technology for microbiologists. Continuing improvements in cytometer instrumentation and in the available range of fluorescent dyes and molecular probes are making it possible to determine (qualitatively and quantitatively) the identities, viability, specific functional activities and overall physiology of microbes. Advances in the range of fluorogenic substrates available for differentiating microbial species, plus combination of fluorescent tagging with specific genome sequence, allow for highly accurate and sensitive detection of microorganisms in food and beverage samples - including those that are non-culturable but which have potential for spoilage or pathogenicity. ..."
From: Attfield P et al (1999) Australasian Biotechnology 9: 159-166

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Flow Cytometers

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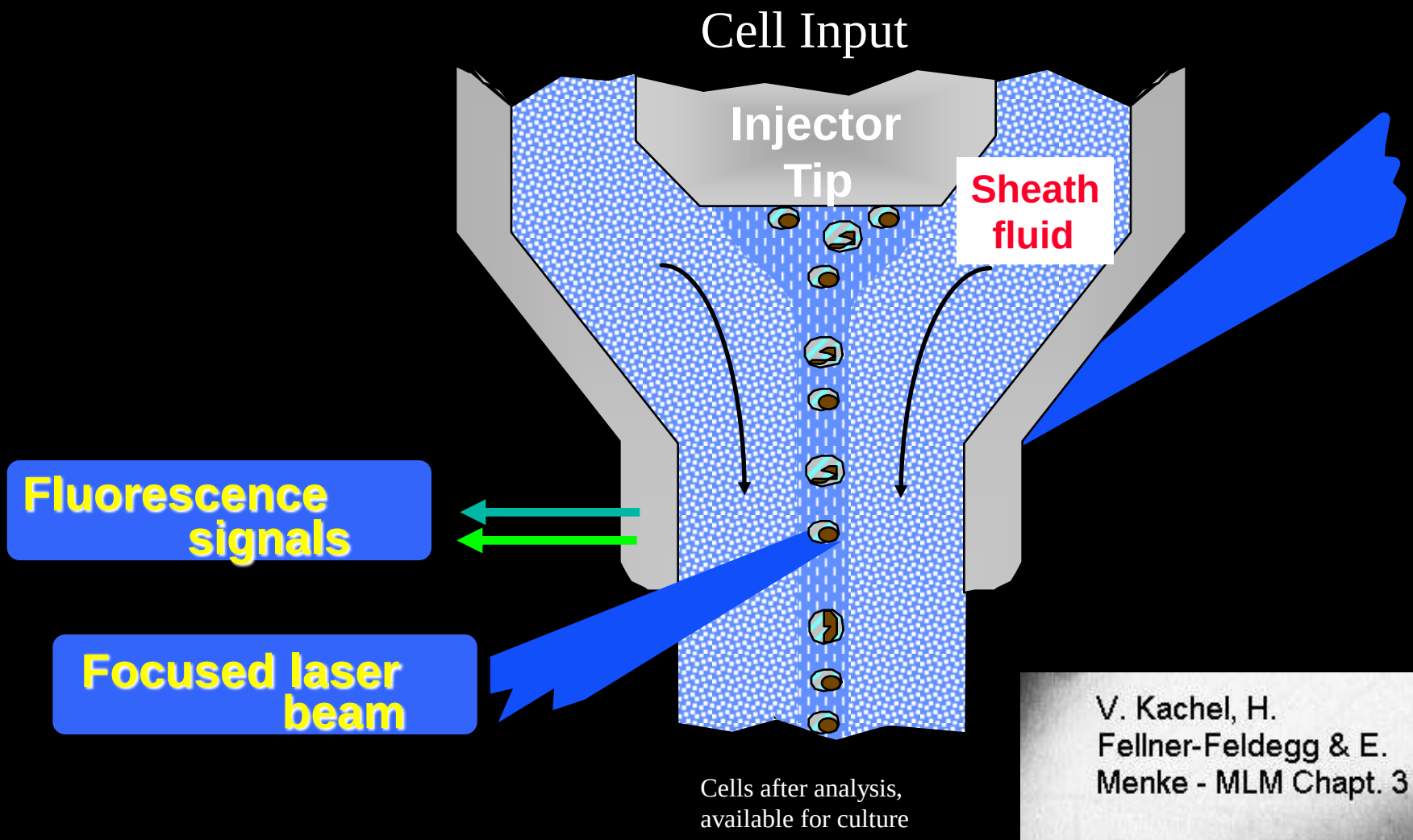
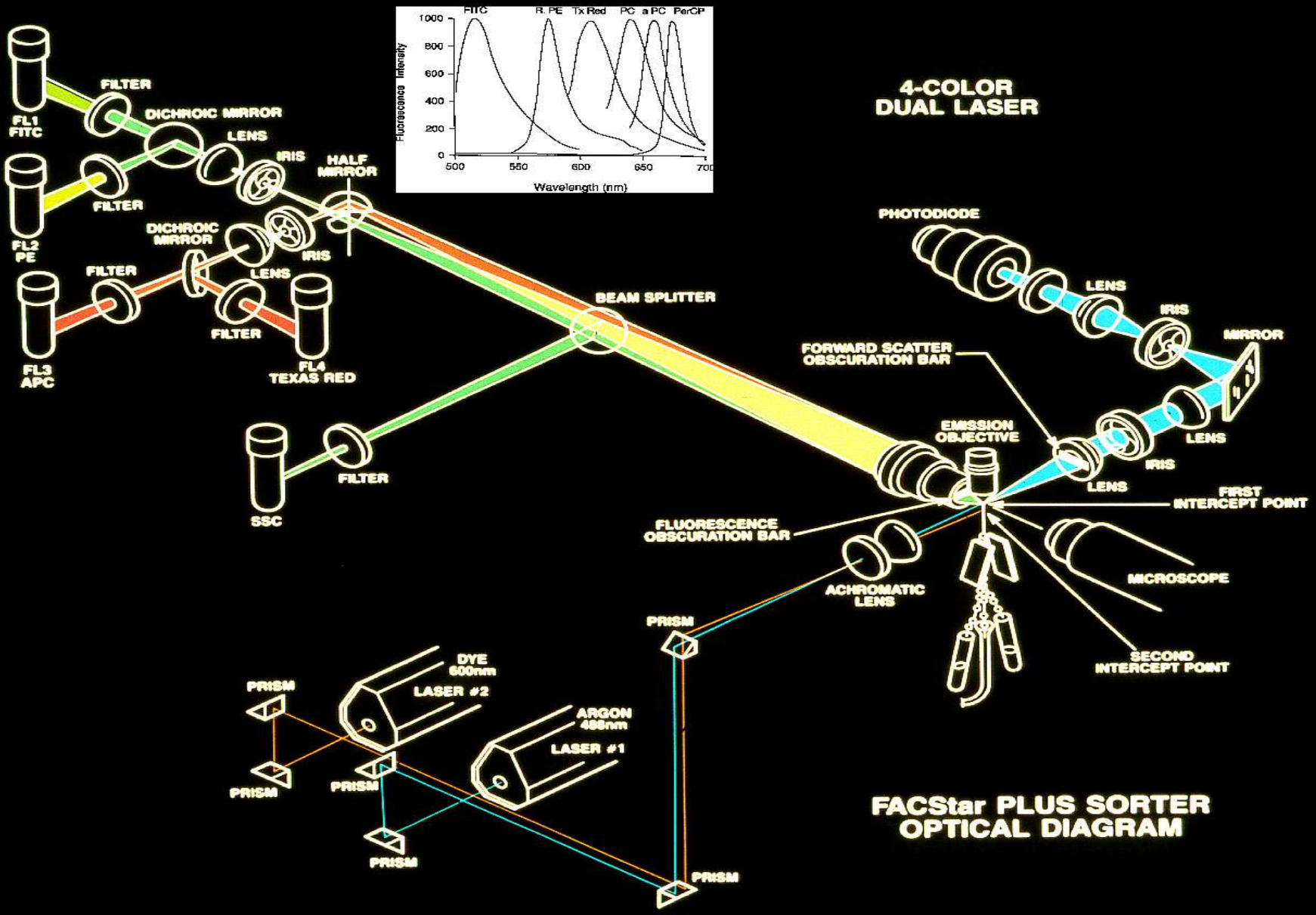
Flow Cytometer Fluidics



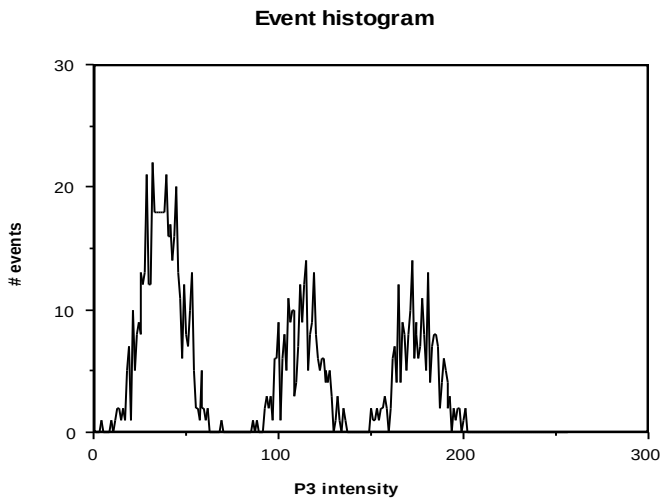
CD-ROM Vol 3 Purdue University Cytometry Laboratories

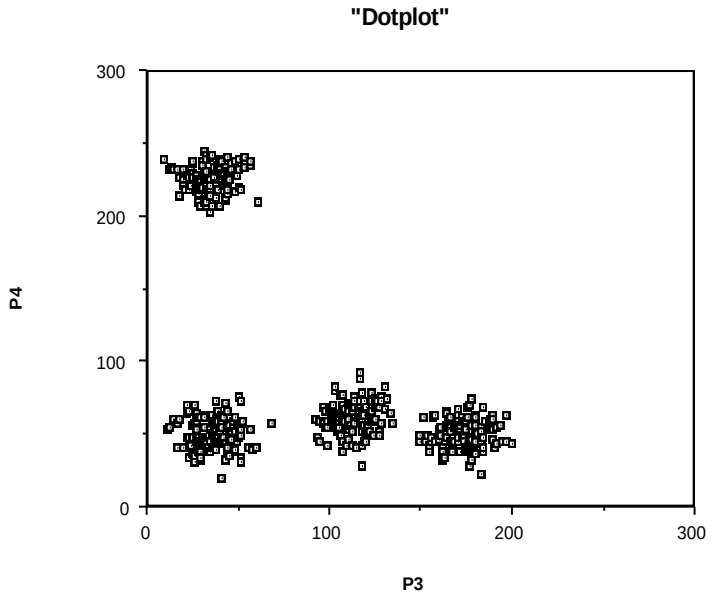
Flow Cytometer Optics



Flow Cytometer Data

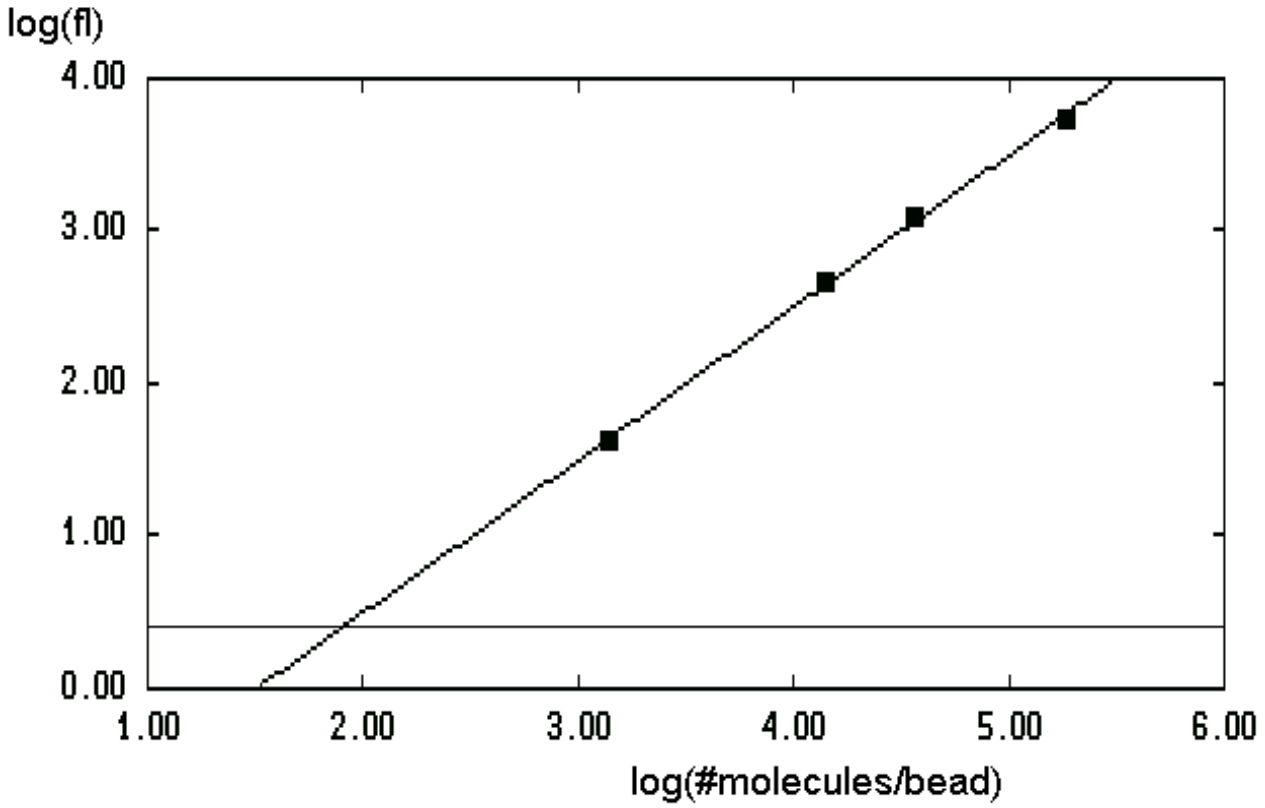
Cell	P1	P2	P3	P4	P5	Pop#
1	242	135	704	175	612	1
2	146	132	690	178	566	1
3	269	147	89	206	580	3
4	442	143	399	250	255	4
5	212	167	155	926	526	2
6	269	2	659	207	575	1
7	204	232	112	171	679	3
8	152	74	160	828	532	2
			…			
9997	215	119	138	936	662	2
9998	244	50	72	261	543	3
9999	214	137	174	1014	597	2
10000	312	87	110	904	560	2





Limit of Detection for Particle Fluorescence

System background at less than 100 fluorescent molecules per particle



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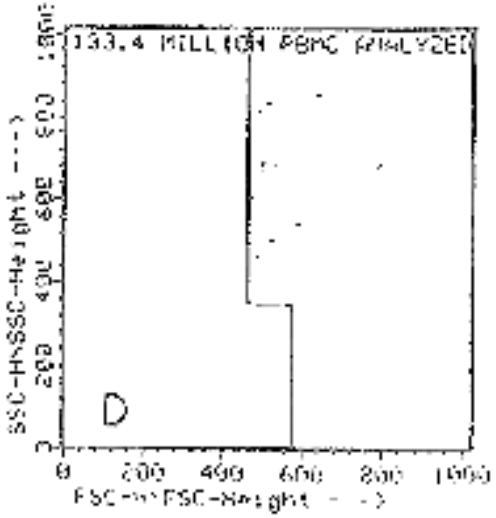
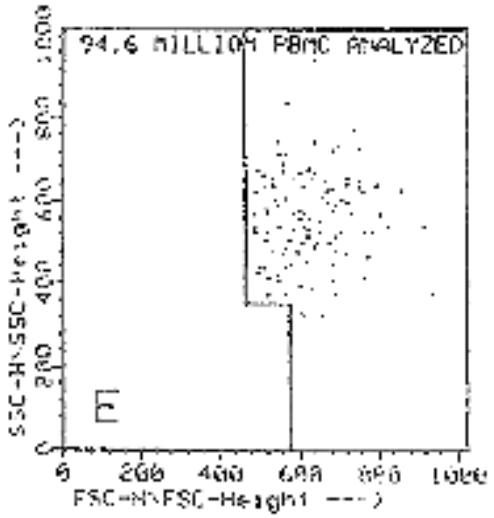
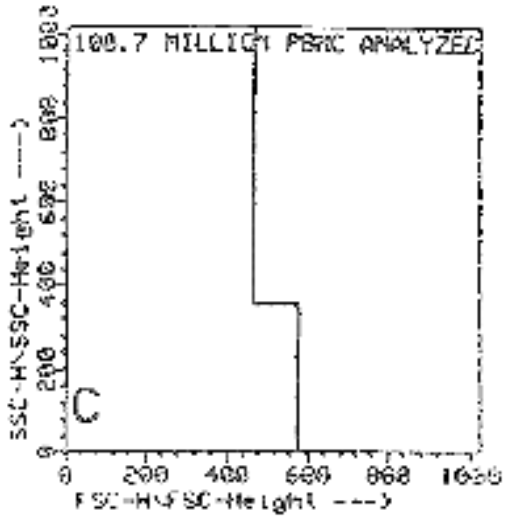
Limit of Detection for Rare Cells



10^{-6}



10^{-5}



control

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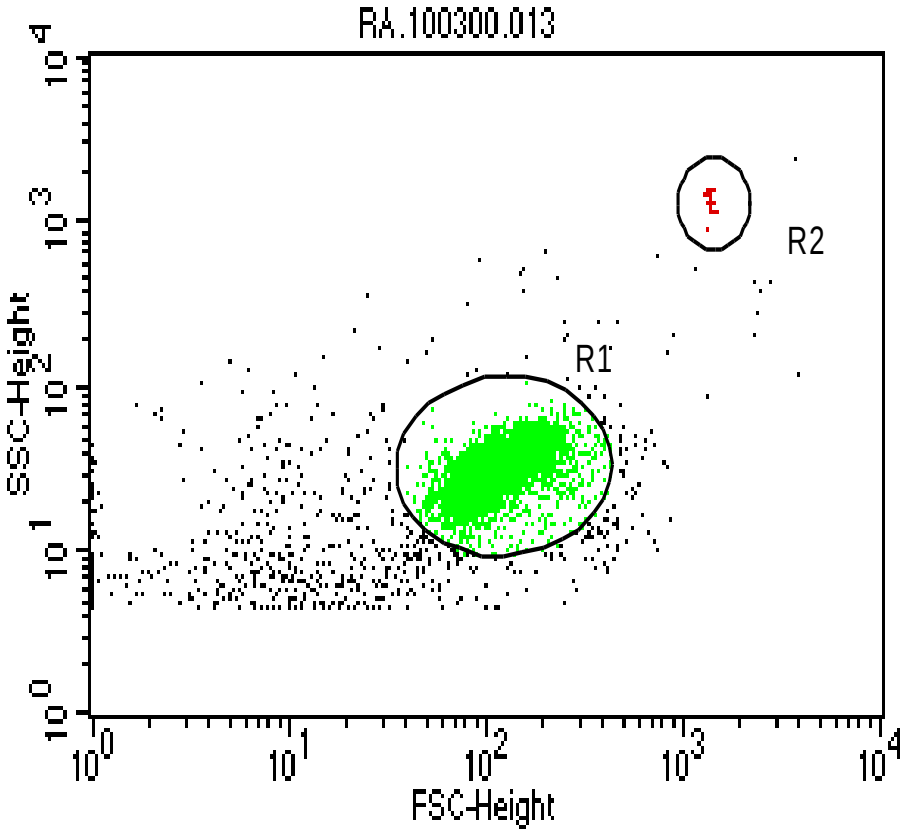
Gross HJ et al, Cytometry. 1993;14(5):519-26

Counting of Bacteria, Based on Light Scatter



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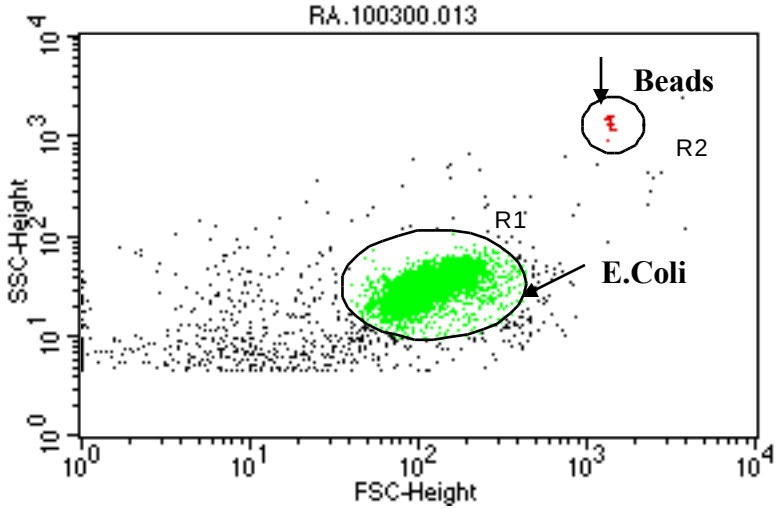
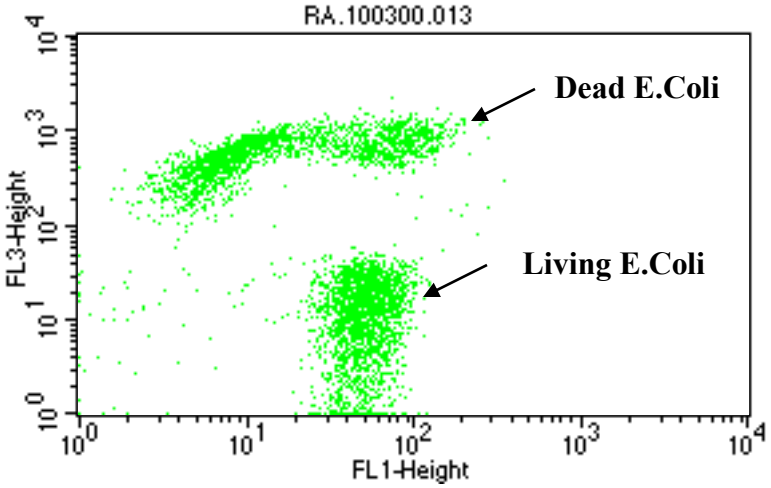
Counting of Bacteria, Based on Nucleic Acid Dye Fluorescence (identification of live/dead cells)





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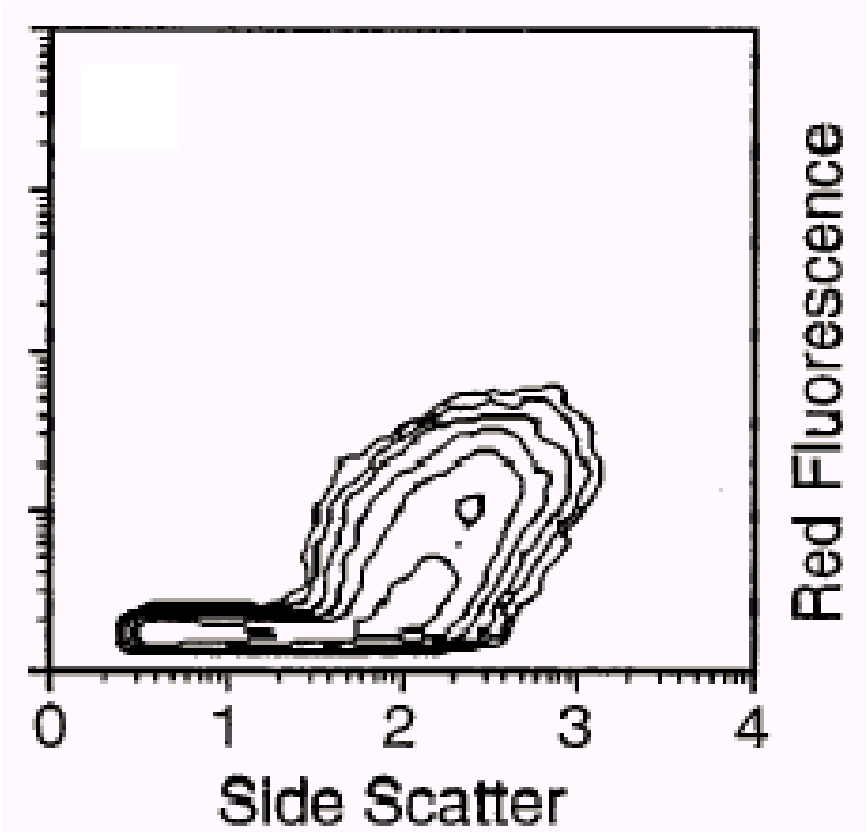
Counting of Bacteria, Based on Fluorogenic Substrates



Reduction of 5-Cyano-2,3-Ditolyl-Tetrazolium by Marine Bacteria

Sideracki ME et al.: AEM 65(6):2409-17 (1999)

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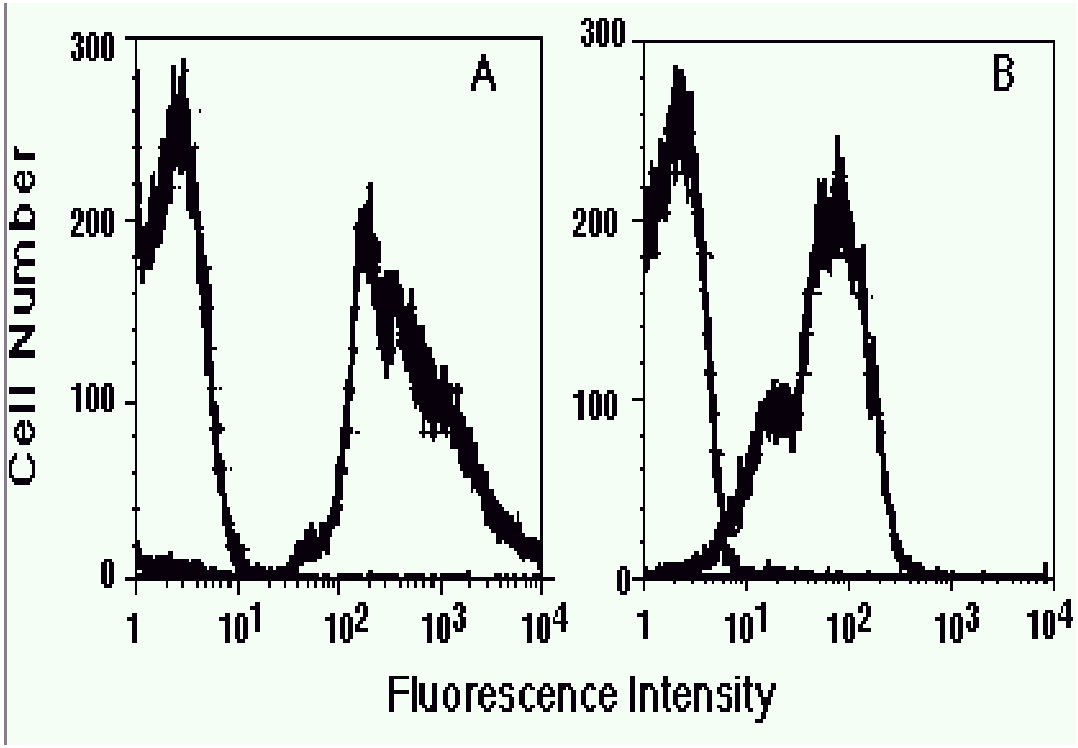
Identification and Counting of Bacteria with Specific Antibodies



Neisseria meningitidis serotypes. Flow cytometric analysis of gram-negative bacterial cells. Dave Duncan, Peggy Ooi, and Robert Zagursky , Purdue Cytometry CD-ROM Series

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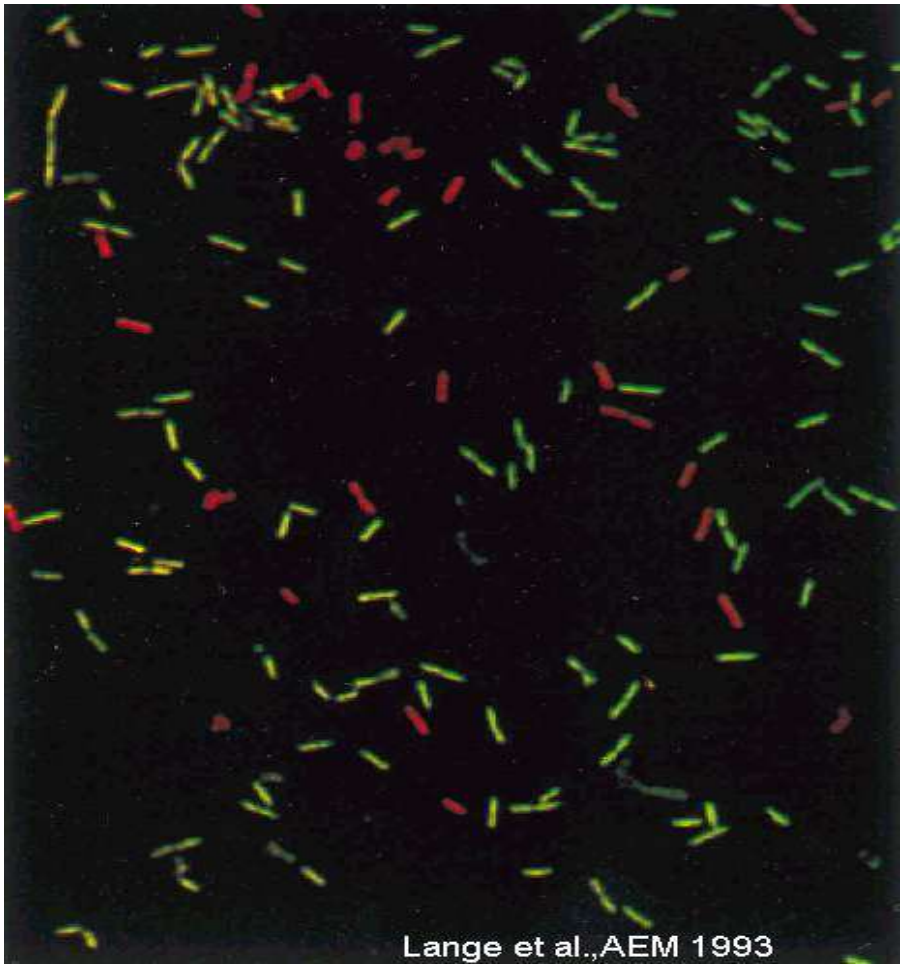
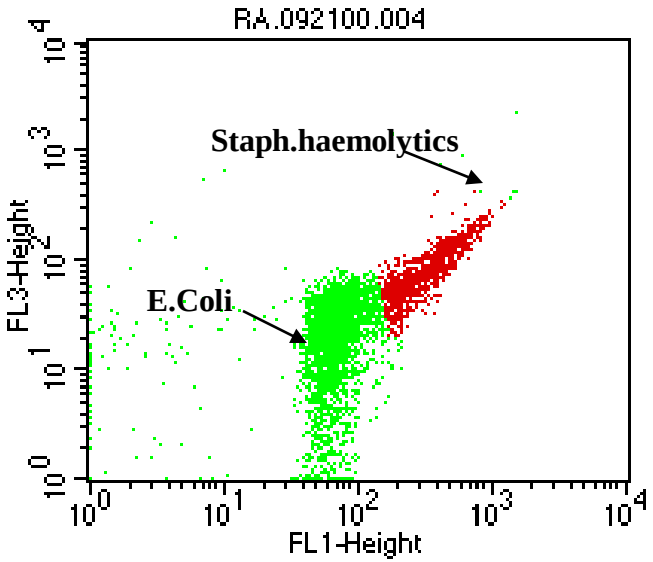
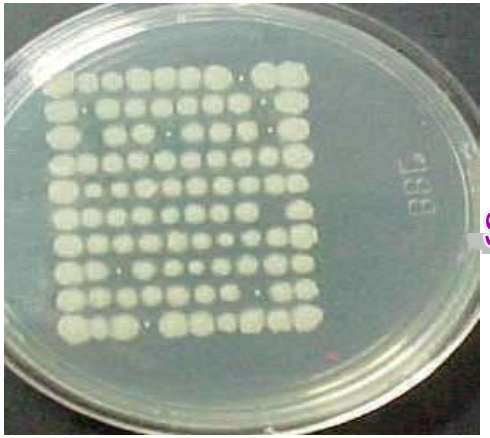
Identification and Counting of Bacteria with Specific Nucleic Acid Probes



P.aeruginosa & E.coli

Combining Flow Cytometry with Culture Methods





Sorted E.Coli colonies

Sorted Staph colonies

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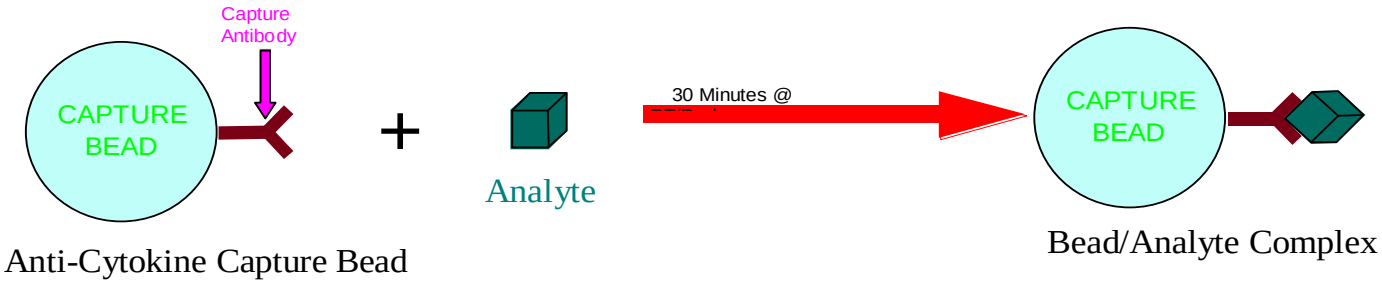
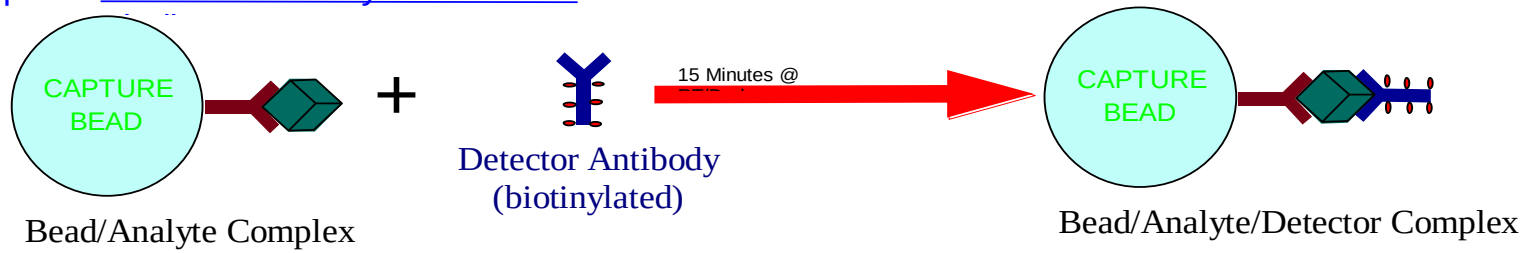
Multiplexed Microbial Identification through Organism Specific Antigens (1)

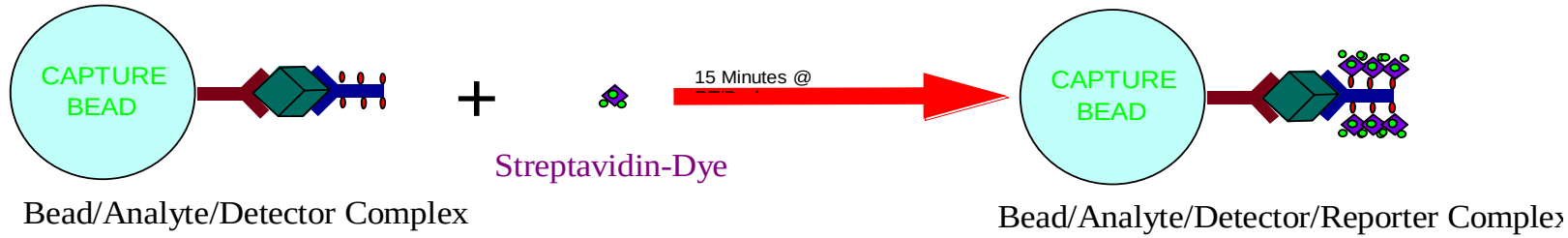
Step 1: Analyte



Step 2: Detector Antibody



Step 3: Reporter Molecule



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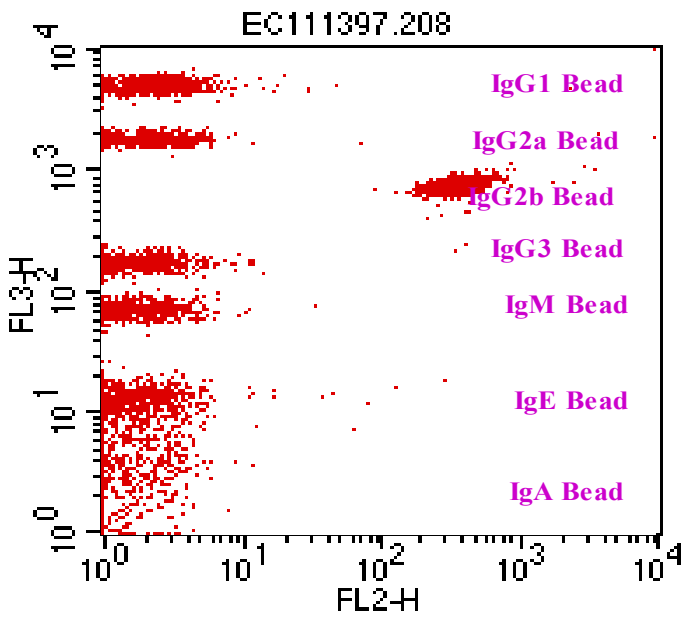
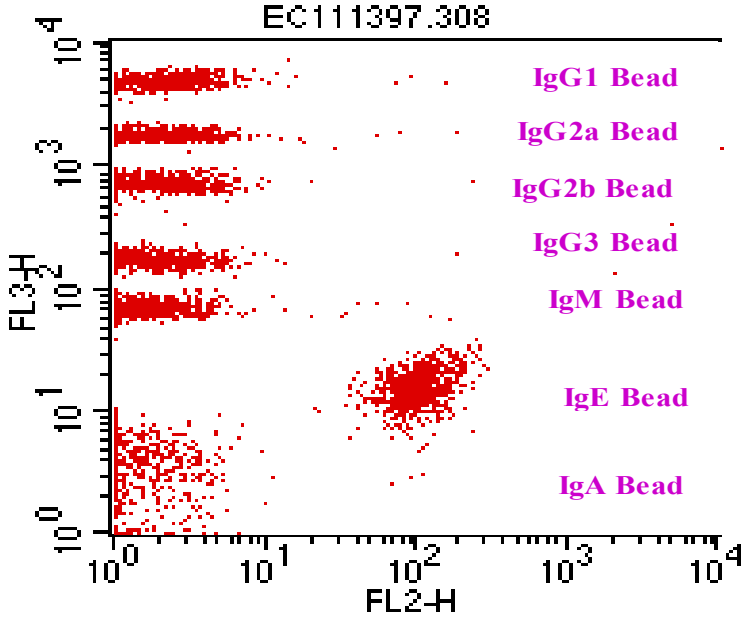
Multiplexed Microbial Identification through Organism Specific Antigens (2)

IgG2b 20 ng



IgE 20 ng



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Microbial Identification by Genetic Fingerprinting

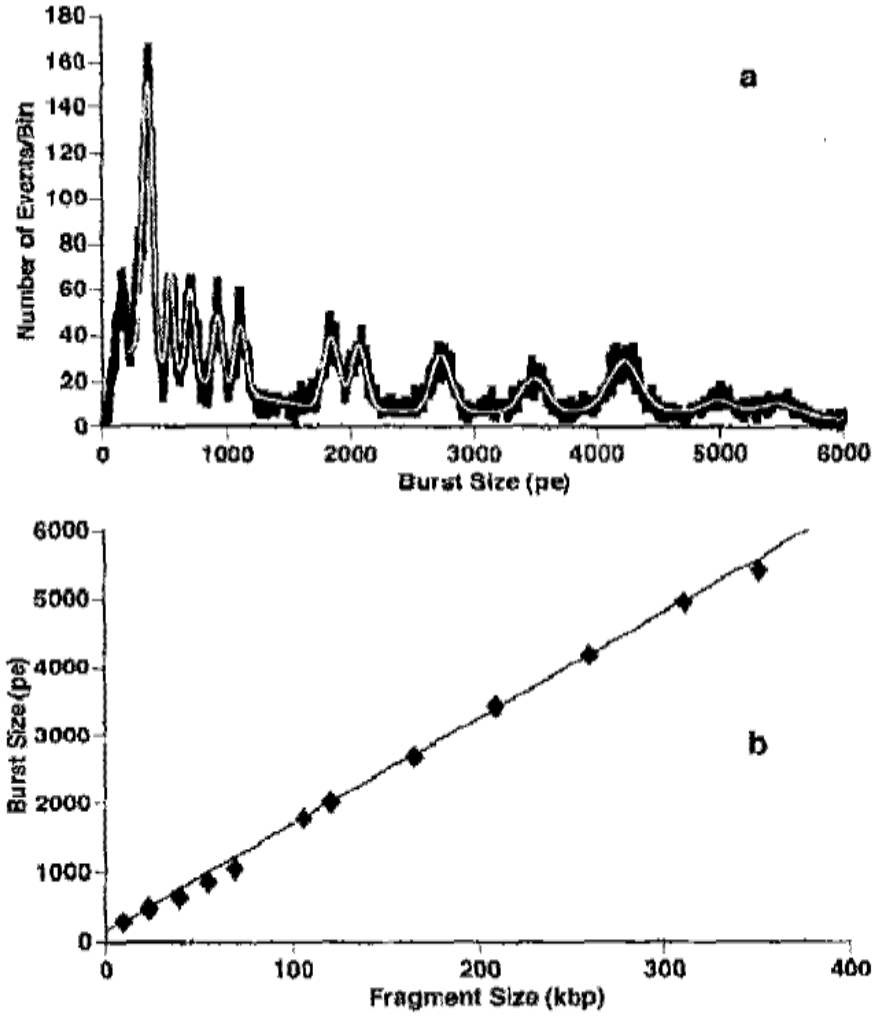

FIG. 2. a: Histogram of the fluorescence burst sizes of *SmaI* digestion of *S. aureus* DNA. Bin width was 10 pe.

From: Huang Z et al. in Cytometry 35: 169-175 (1999)

Application examples

- Hygiene monitoring
- Enumeration of live/dead micro-organisms for microcide efficacy
- Enumeration of bacteria in milk
- ...

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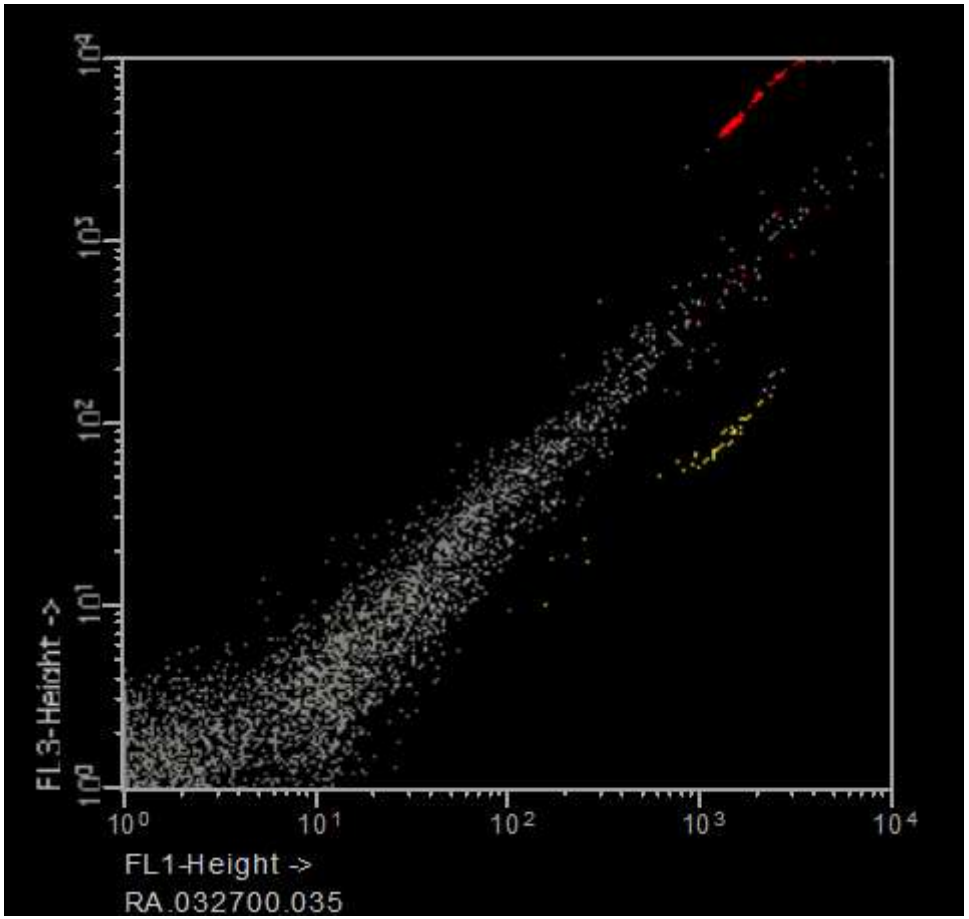
Detection (Hygiene Monitoring)

Procedure:

- add dye solution to sample
- incubate for 1 min
- analyse



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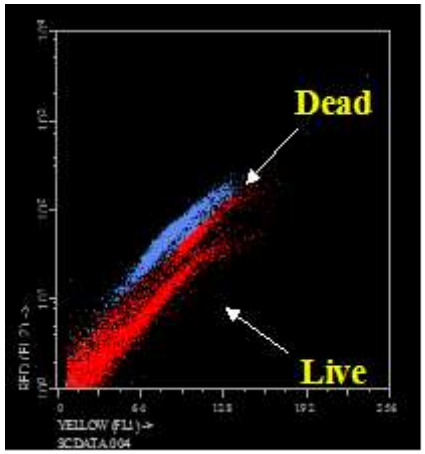
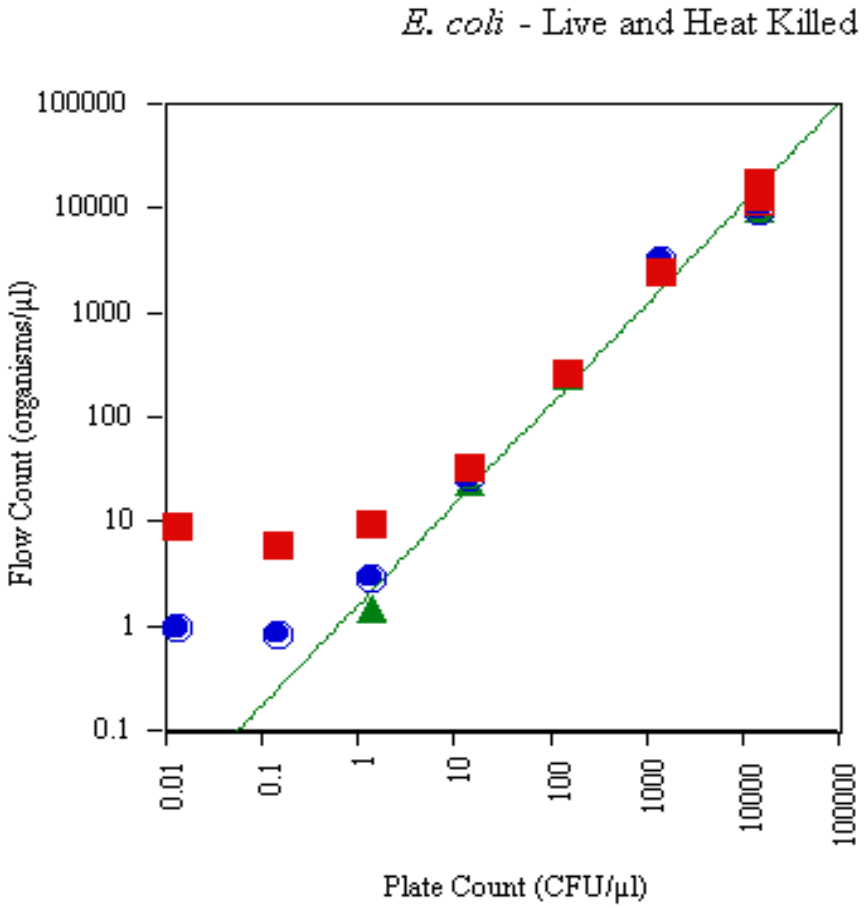
Detection (live/dead discrimination)

Procedure:

- add dye solution to sample
- incubate for 1 min
- analyse





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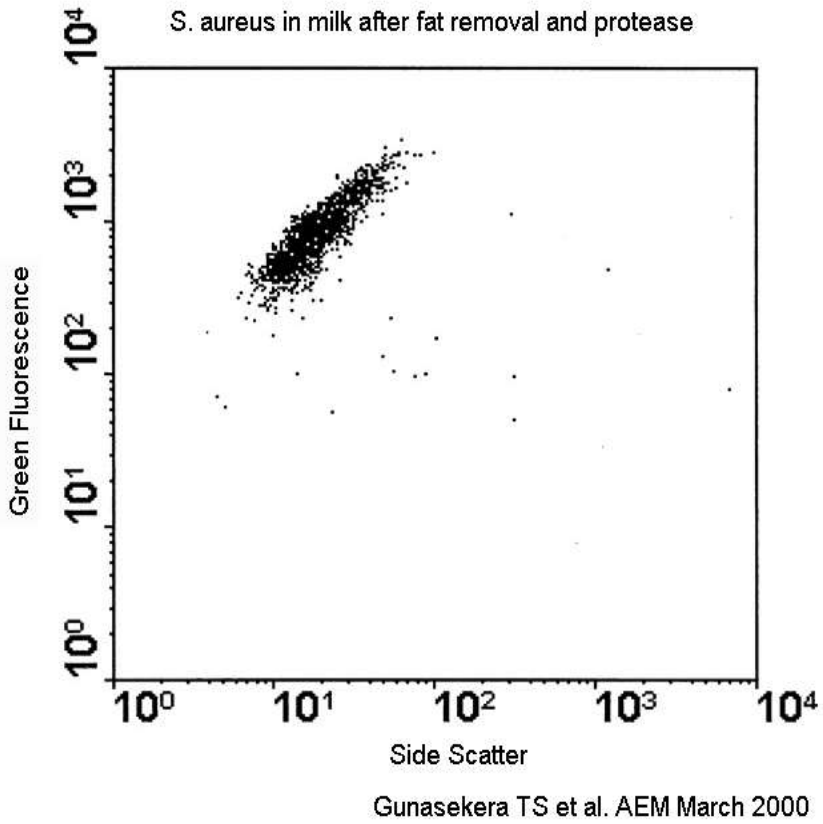
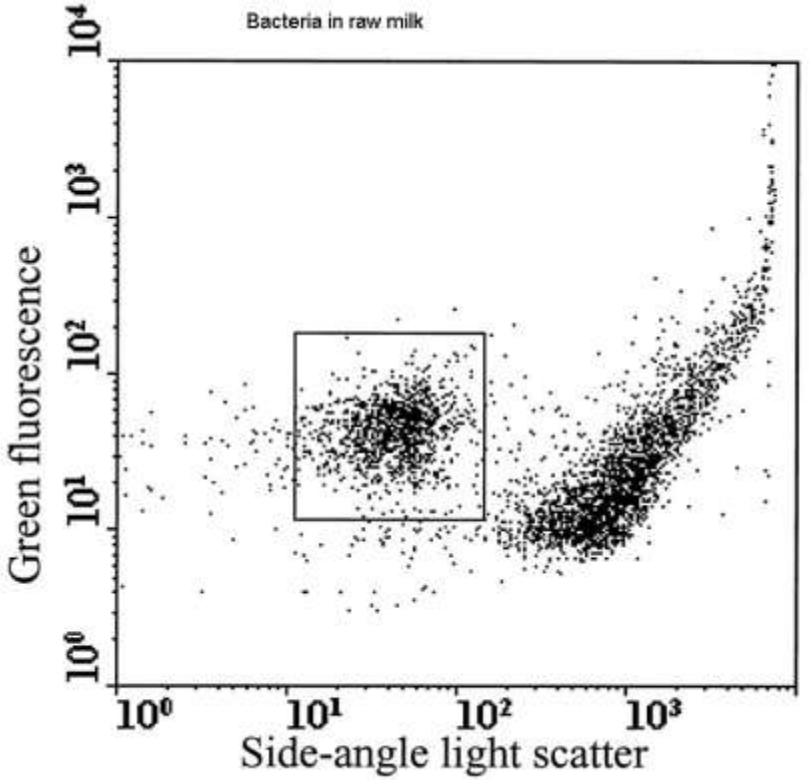
Bacteria in Milk



Gunasekera TS et al. AEM March 2000



Gunasekera TS et al. AEM March 2000

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Criteria for Comparison of Methods

- Sensitivity & Specificity
- Time to Result
- Ease of Use
- Cost per Test (Instrument, Reagents, Labor)
- Laboratory Space Requirements

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Conclusions about Flow Cytometry for Food Microbiology

- High sensitivity
- Wide dynamic range (10^3 to 10^7 mL^{-1})
- High analysis rates to 10^4 particles sec^{-1}
- High specificity with antibodies or NA probes
- Live/dead discrimination
- Extremely short time to result
- Viable cells can be re-covered for culture
- Good Ease-of-use
- Moderate cost per test
- Moderate bench space requirement

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End of presentation

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