Bioinformatics Cytometry Context

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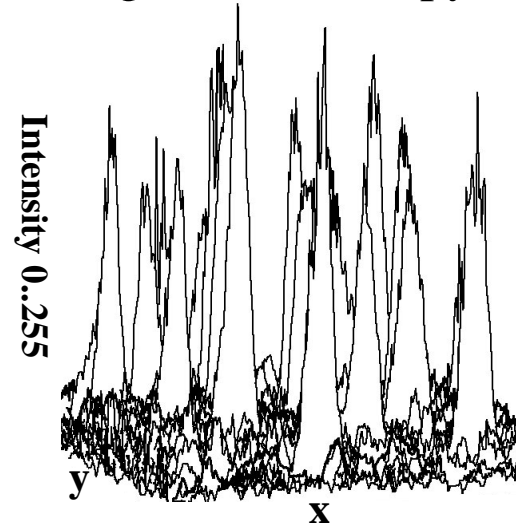# Biology Research Targets and Tools

Organism

NMR

X-ray imaging

Organ

Ultrasound

2-photon imaging

Tissue

In-vivo cytometry

Light microscopy

Single Cell

Electron microscopy

Flow cytometry

Organelle

Cell imaging

NA sequencing

Macromolecule

Mass spectrometry

TIRF microscopy

Small molecules

Electrophoresis

Contrast agents

Affinity reagents
- antibodies
- probes

Enzyme substrates

Labels
- absorbance
- fluorescence
- element tags

…

Sample prep

Cytometry Data

Flow Cytometry

Numbers in Memory

FSC

SSC

FITC

← Laser delay →

APC
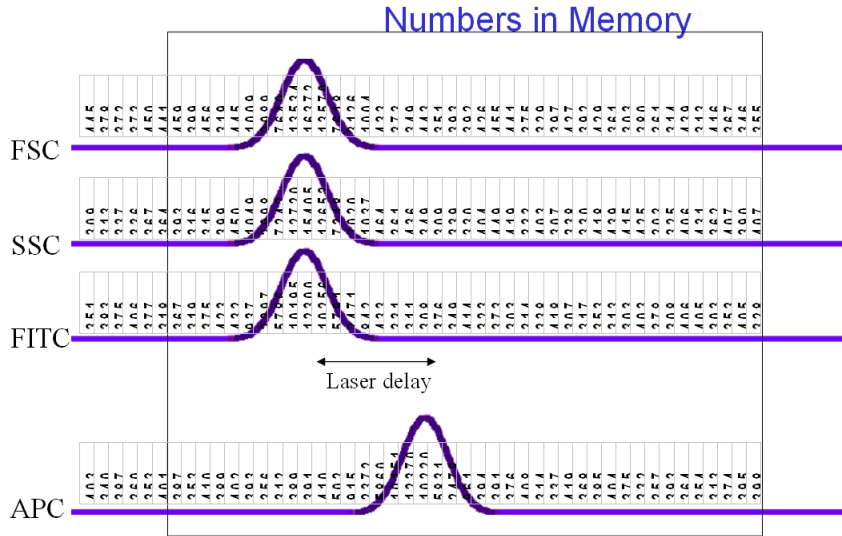
Digital microscopy

Intensity 0..255

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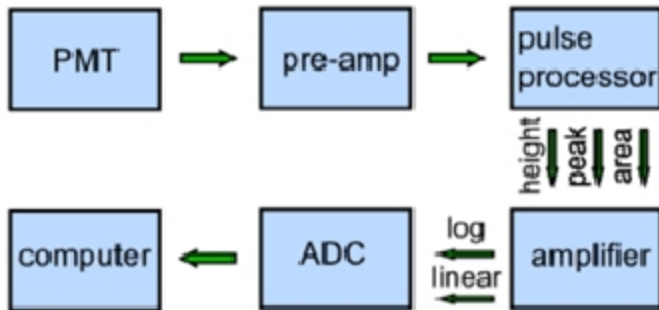
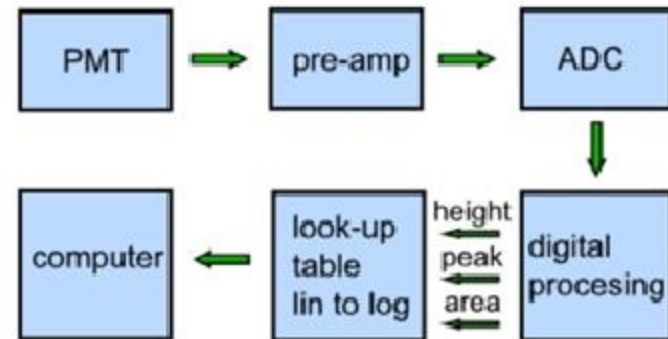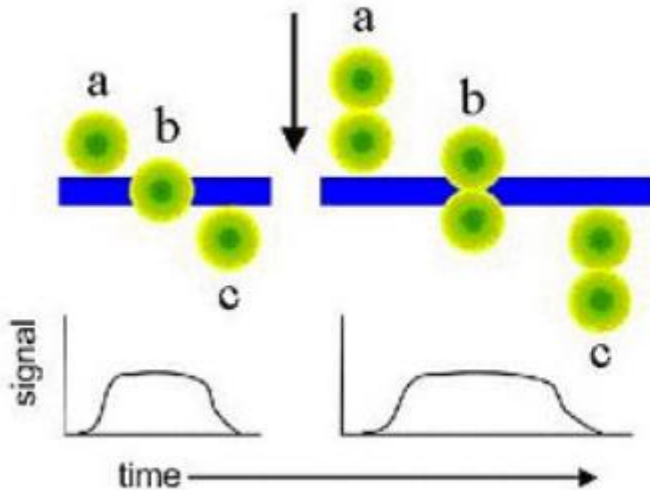| Cell	P1	P2	P3	P4	P5	Pop#
1	242	135	704	175	612	1
2	146	132	690	178	566	1
3	269	147	89	206	580	3
4	442	143	399	250	255	4
5	212	167	155	926	526	2
6	269	2	659	207	575	1
7	204	232	112	171	679	3
8	152	74	160	828	532	2
			…			
9997	215	119	138	936	662	2
9998	244	50	72	261	543	3
9999	214	137	174	1014	597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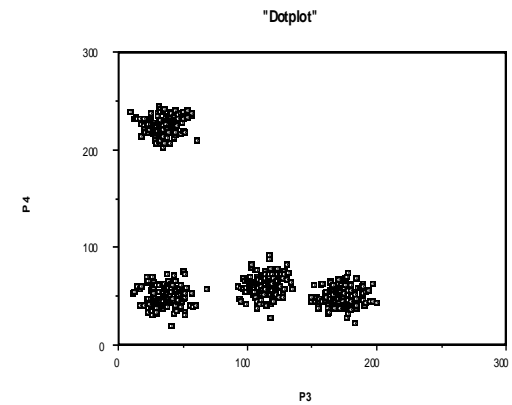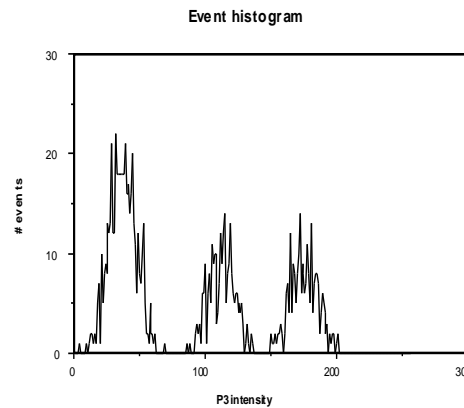
Event histogram

events

P3 intensity

"Dotplot"

P4

P3

Signal Processing
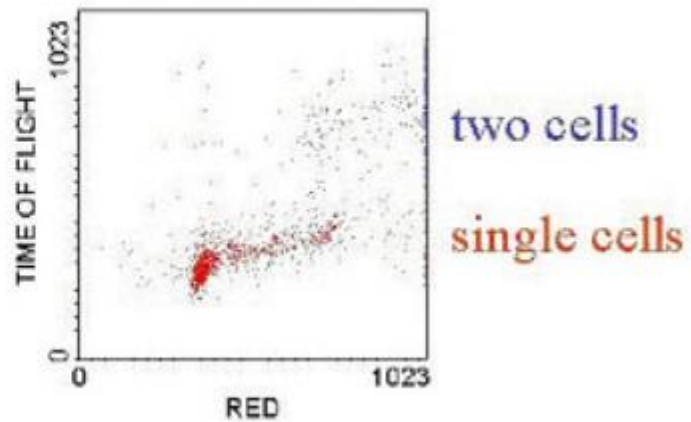
Data File Standards for Cytometry

Data File Standard forFlow Cytometry, Version FCS3.0

Data File Standards Committeeof the International Society for Analytical Cytology (ISAC)

FCS File Segments
- Header
- Text
- Data
- Analysis
- CRC value

With pre-determined and user defined keywords in text section

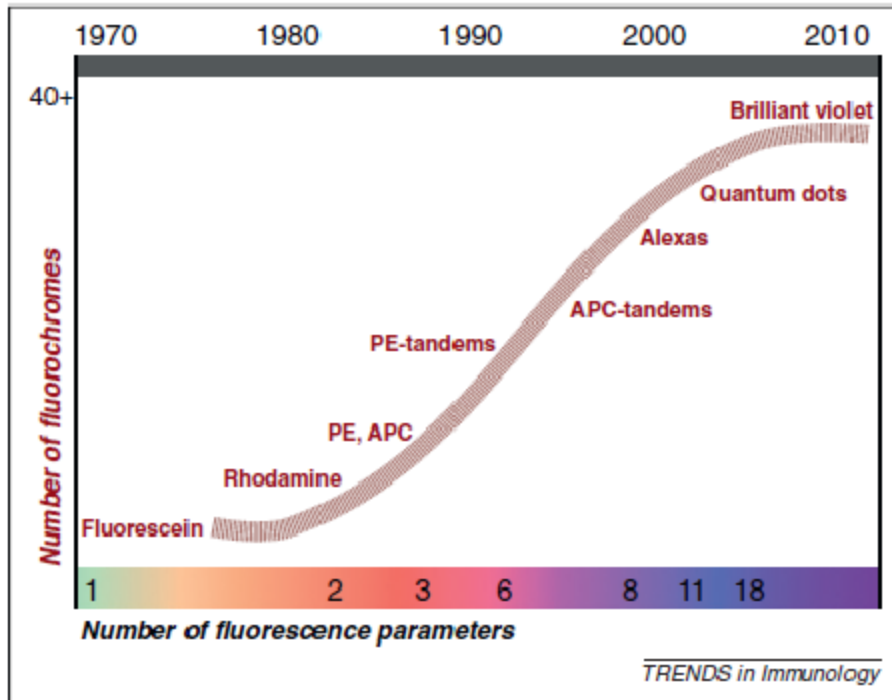ISAC Recommendation   MIFlowCyt - Minimum Information about a Flow Cytometry Experiment

Minimum Information about a Flow Cytometry Experiment

MIFlowCyt 1.0

A standard for outlining the minimum information required to report the experimental details of flow cytometry experiments

Other file standards for cytometry measurements.

Labels for Multi-parameter Analysis

Figure 1. History of fluorochrome development. A timeline showing when the major fluorochromes were introduced, and how this related to the maximum number of parameters that could be simultaneously measured at that time.

Brendall SC et. al. (2012) Trends Immunol

Examples: Data Analysis/Display Methods

Bendall SC et al. (2012) *TRENDS in Immunology*
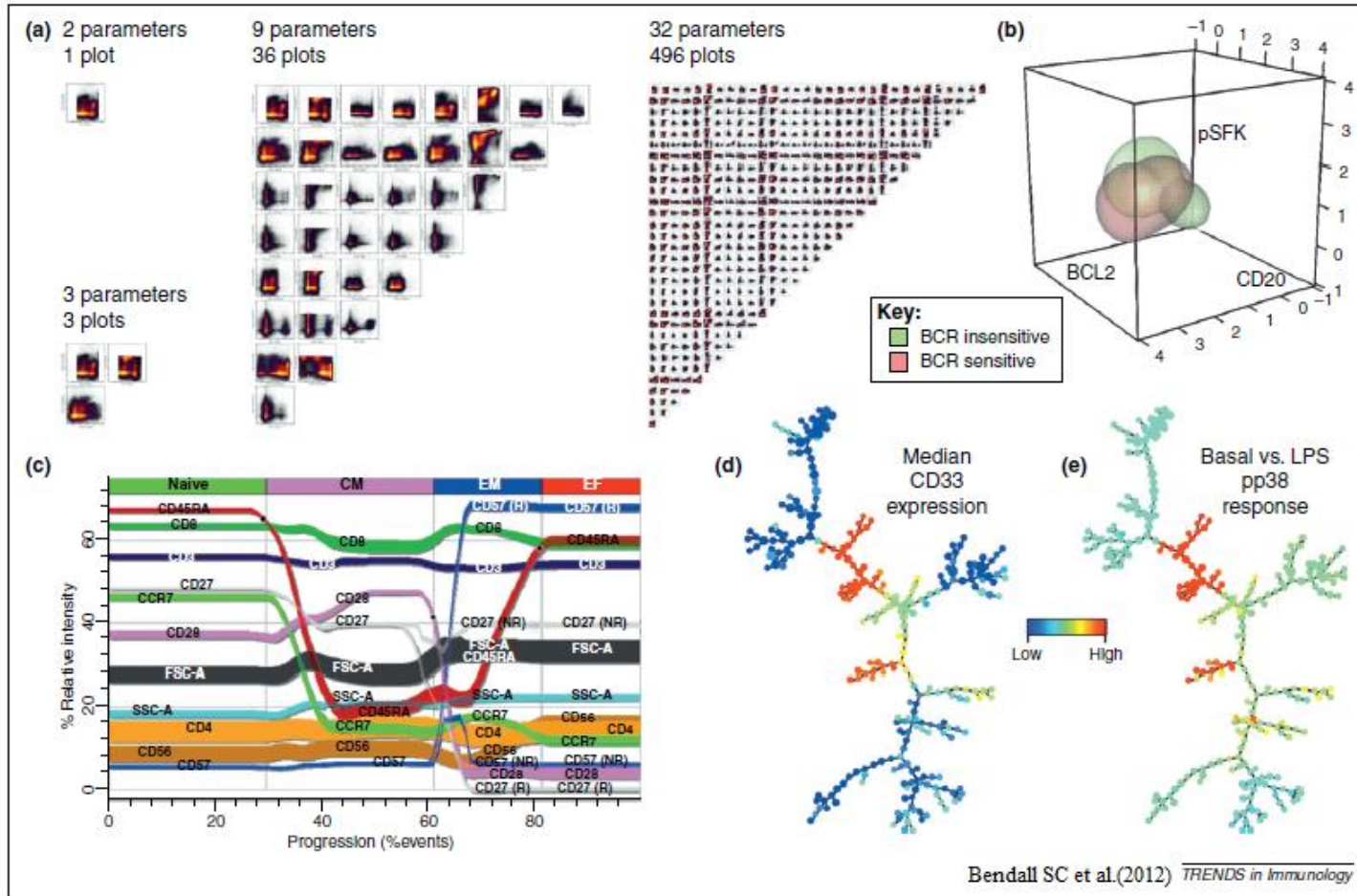
Important work:

Critical assessment of automated flow cytometry data analysis techniques

Nima Aghaeepour[1], Greg Finak[2], The FlowCAP Consortium[3], The DREAM Consortium[3], Holger Hoos[4], Tim R Mosmann[5], Ryan Brinkman[1,7], Raphael Gottardo[2,7] & Richard H Scheuermann[6,7]

Summary: Information Flow

Pre-measurement:

Cells/tissues/organisms, Reagents, Controls, Intended instrument parameters, Nature of prepared samples, …

Measurement

Data from measurement including actual instrument conditions e.g. fcs files

Post-measurement

Data analysis with appropriate information extraction algorithms e.g. gating or cluster analysis, integration of information with other data sources e.g. MS. Global information analysis e.g. comparison of normal vs. diseased

Integration of information from other technologies is important for a comprehensive analysis of a system.
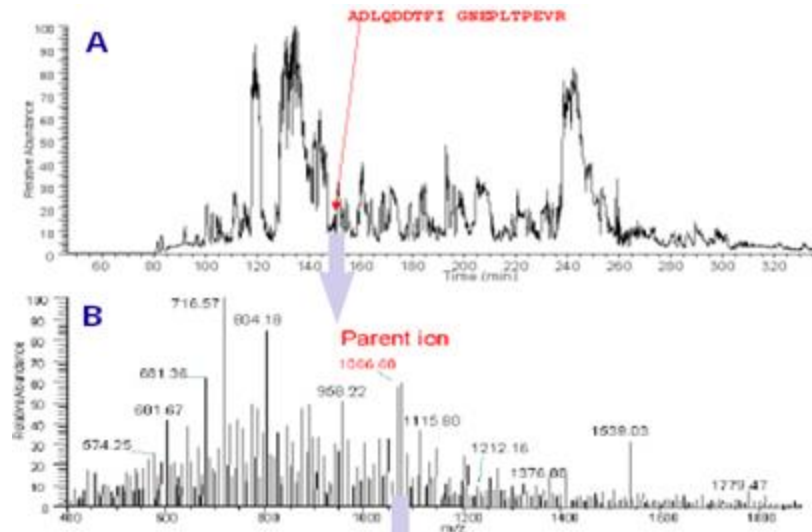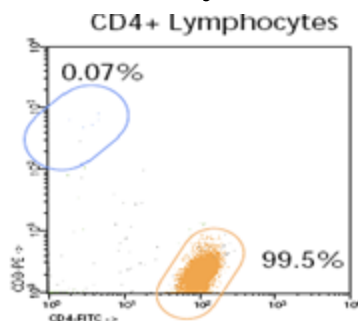
Example: Cell Signaling in Oncology

1) Access to samples
   - ideally uniform initial therapy
   - ideally long term clinical outcomes or paired samples
   - ideally balanced training and testing sample sets
2) Flow cytometry & signaling network profiles
   - map signaling in every cell within a tumor specimen
   - markers for tumor, non-malignant, and cell subsets
   - cell sorting for follow up studies of genetics and epigenetics
3) Cloud computing to link all our knowledge & tools
   - data storage & annotation, data sharing
   - web based analysis tools for researchers
   - computational analysis & modeling tools (SPADE)
   - informatics (patient information, ontologies)

provided by Nikesh Kotecha, Cytobank

The experts in this workshop will teach advanced algorithms for data analysis and software for integrating results from cytometric measurements with other data for a comprehensive analysis of biological systems.

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