



Intravital Microscopy, Flow Cytometry and Cell Sorting

7 – 13 July 2013 | Berlin, Germany

Bioinformatics Cytometry Context

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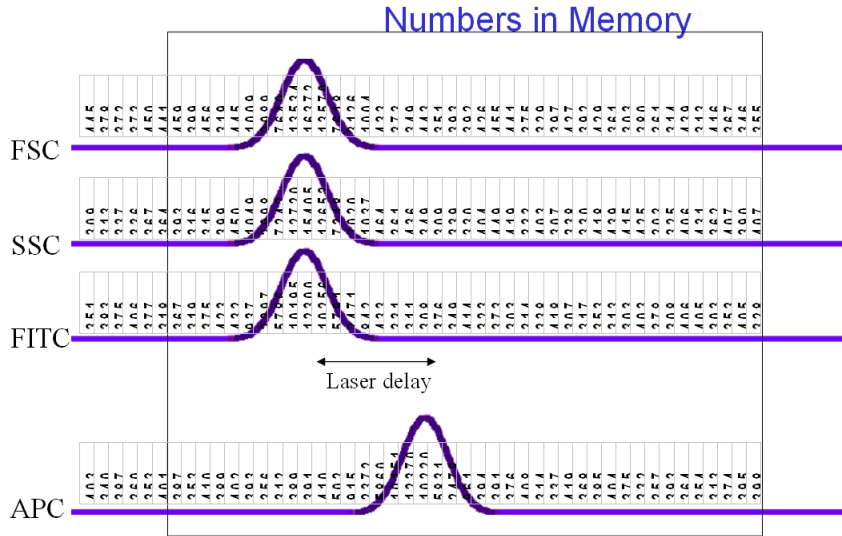
<http://www.desatoya.com>

Biology Research Targets and Tools

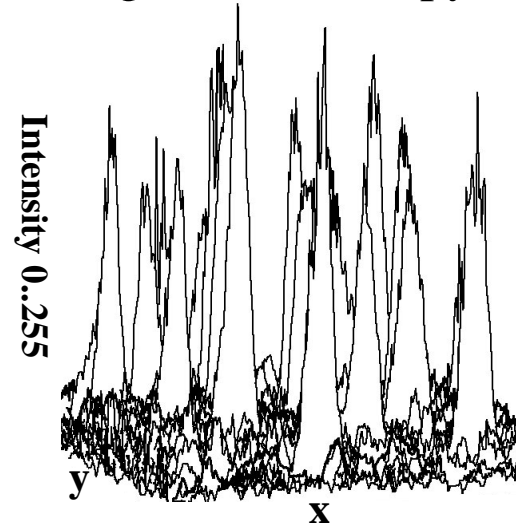
| | | |
|------------------------|--------------------------|-------------------|
| Organism | NMR | Contrast agents |
| | X-ray imaging | Affinity reagents |
| Organ | Ultrasound | - antibodies |
| | <u>2-photon imaging</u> | - probes |
| Tissue | <u>In-vivo cytometry</u> | Enzyme substrates |
| | Light microscopy | Labels |
| Single Cell | Electron microscopy | - absorbance |
| | <u>Flow cytometry</u> | - fluorescence |
| Organelle | <u>Cell imaging</u> | - element tags |
| | NA sequencing | ... |
| Macromolecule | Mass spectrometry | |
| | TIRF microscopy | |
| Small molecules | Electrophoresis | Sample prep |

Cytometry Data

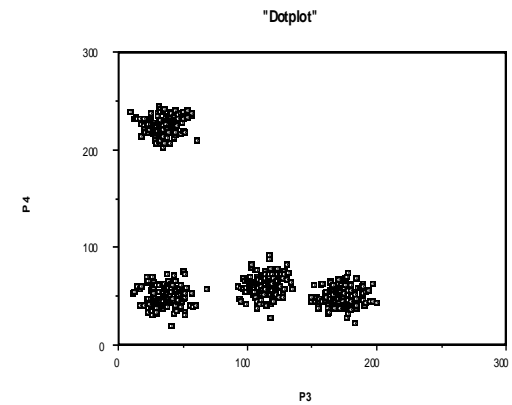
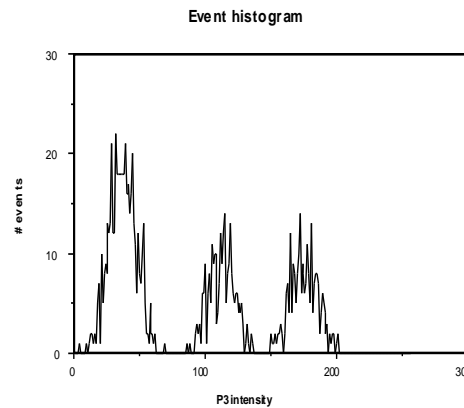
Flow Cytometry



Digital microscopy

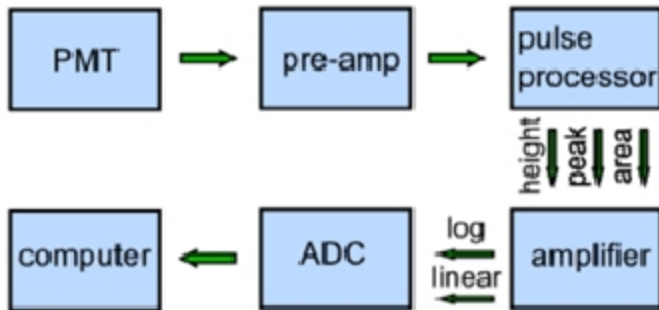


| Cell | P1 | P2 | P3 | P4 | P5 | Pop# |
|-------|-----|-----|-----|------|-----|------|
| 1 | 242 | 135 | 704 | 175 | 612 | 1 |
| 2 | 146 | 132 | 690 | 178 | 566 | 1 |
| 3 | 269 | 147 | 89 | 206 | 580 | 3 |
| 4 | 442 | 143 | 399 | 250 | 255 | 4 |
| 5 | 212 | 167 | 155 | 926 | 526 | 2 |
| 6 | 269 | 2 | 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 |



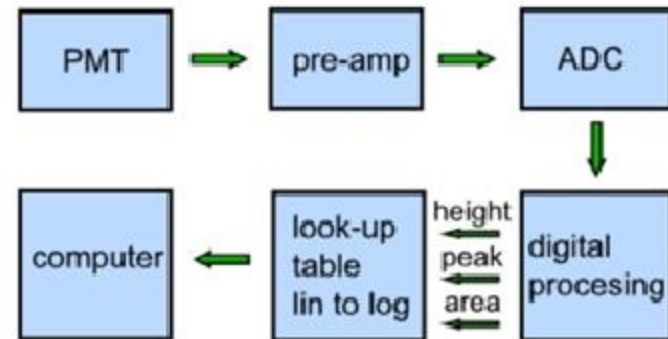
Signal Processing

analogue

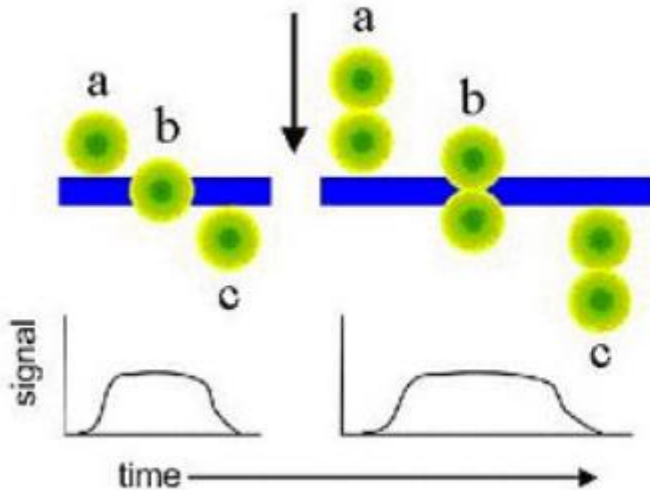


http://flowbook.denovosoftware.com/@api/deki/files/21/=Chapter_02

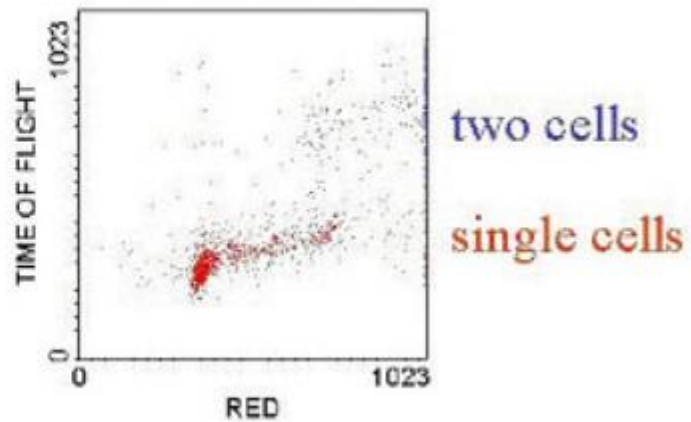
digital



http://flowbook.denovosoftware.com/@api/deki/files/21/=Chapter_02



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Data File Standards for Cytometry

7/5/13

FCS3.0

Data File Standard for Flow Cytometry, Version FCS3.0

Data File Standards Committee of 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

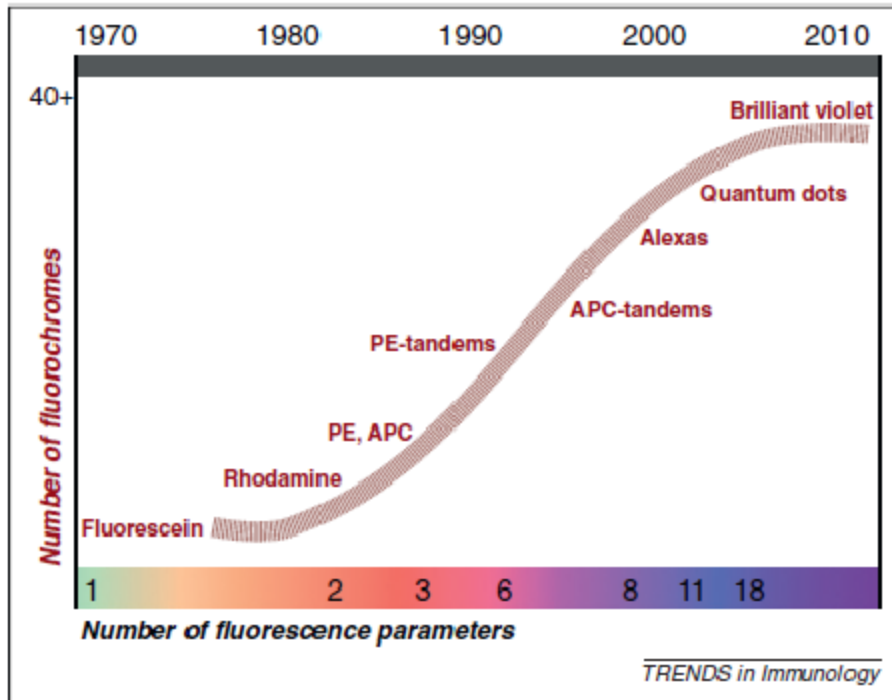
Minimum Information about a Flow Cytometry Experiment

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



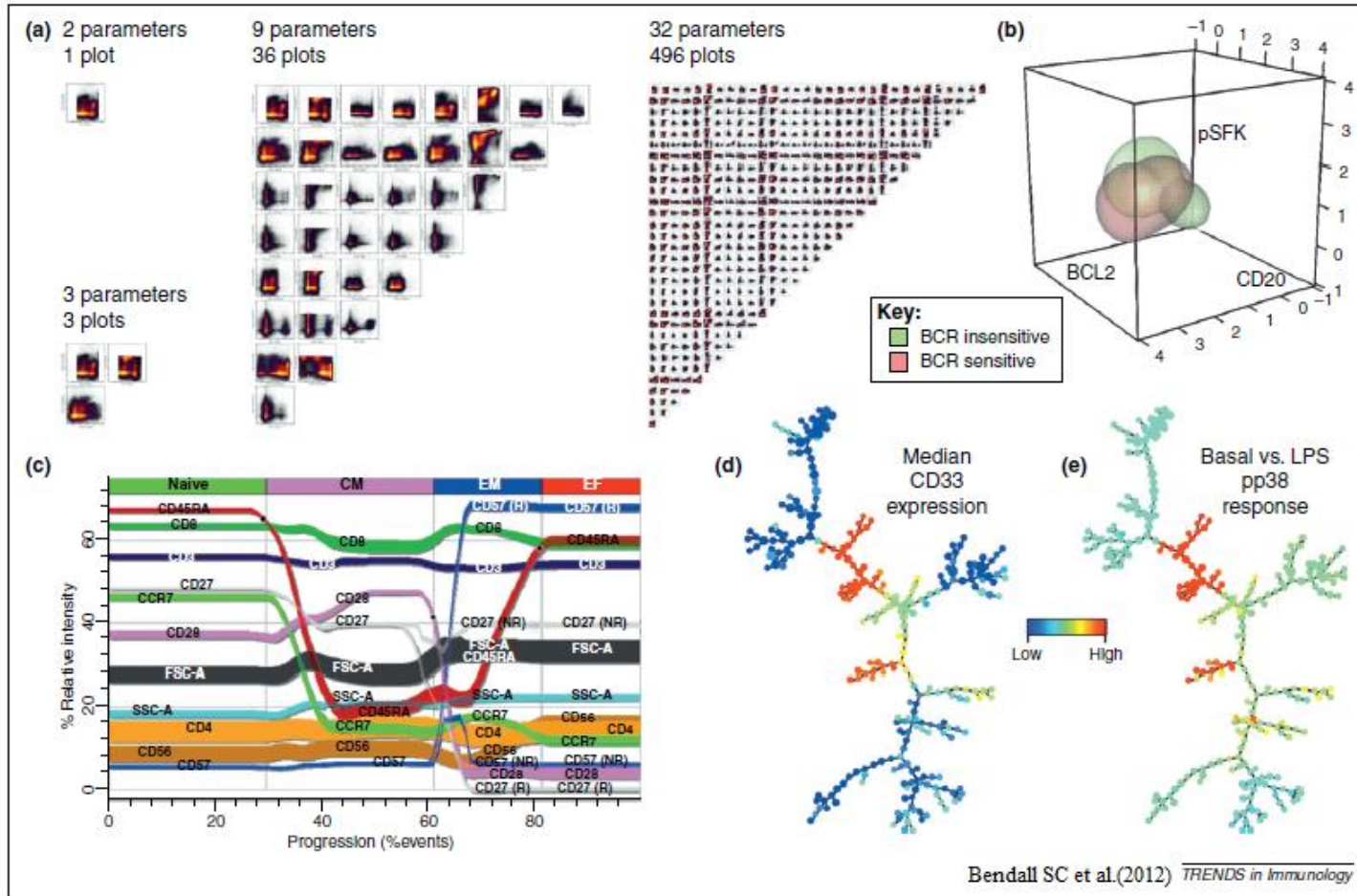
| | | | | | | | | | | | | | | | | | |
|----------------------|-----------------------|----------------------|----------------------------|----------------------|--------------------------|------------------------|------------------------|-------------------------|--------------------------|--------------------------|------------------------|-------------------------|-------------------------|-----------------------|--------------------------|-----------------------|-------------------------|
| 1 H Hydrogen | | | | | | | | | | | | | | | | | 2 He Helium |
| 3 Li Lithium | 4 Be Beryllium | | | | | | | | | | | 5 B Boron | 6 C Carbon | 7 N Nitrogen | 8 O Oxygen | 9 F Fluorine | 10 Ne Neon |
| 11 Na Sodium | 12 Mg Magnesium | Biological ICP-MS | | | | | | | | | | 13 Al Aluminum | 14 Si Silicon | 15 P Phosphorus | 16 S Sulfur | 17 Cl Chlorine | 18 Ar Argon |
| 19 K Potassium | 20 Ca Calcium | 21 Sc Scandium | 22 Ti Titanium | 23 V Vanadium | 24 Cr Chromium | 25 Mn Manganese | 26 Fe Iron | 27 Co Cobalt | 28 Ni Nickel | 29 Cu Copper | 30 Zn Zinc | 31 Ga Gallium | 32 Ge Germanium | 33 As Arsenic | 34 Se Selenium | 35 Br Bromine | 36 Kr Krypton |
| 37 Rb Rubidium | 38 Sr Strontium | 39 Y Yttrium | 40 Zr Zirconium | 41 Nb Niobium | 42 Mo Molybdenum | 43 Tc Technetium | 44 Ru Ruthenium | 45 Rh Rhodium | 46 Pd Palladium | 47 Ag Silver | 48 Cd Cadmium | 49 In Indium | 50 Sn Tin | 51 Sb Antimony | 52 Te Tellurium | 53 I Iodine | 54 Xe Xenon |
| 55 Cs Cesium | 56 Ba Barium | * | 72 Hf Hafnium | 73 Ta Tantalum | 74 W Tungsten | 75 Re Rhenium | 76 Os Osmium | 77 Ir Iridium | 78 Pt Platinum | 79 Au Gold | 80 Hg Mercury | 81 Tl Thallium | 82 Pb Lead | 83 Bi Bismuth | 84 Po Polonium | 85 At Astatine | 86 Rn Radon |
| 87 Fr Francium | 88 Ra Radium | ** | 104 Rf Rutherfordium | 105 Db Dubnium | 106 Sg Seaborgium | 107 Bh Bohrium | 108 Hs Hassium | 109 Mt Meitnerium | 110 Uun Ununennium | 111 Uuu Ununennium | 112 Uub Unbibium | 114 Uuq Unquadium | | | | | |
| | | | * 57 La Lanthanum | 58 Ce Cerium | 59 Pr Praseodymium | 60 Nd Neodymium | 61 Pm Promethium | 62 Sm Samarium | 63 Eu Europium | 64 Gd Gadolinium | 65 Tb Terbium | 66 Dy Dysprosium | 67 Ho Holmium | 68 Er Erbium | 69 Tm Thulium | 70 Yb Ytterbium | 71 Lu Lutetium |
| | | | ** 89 Ac Actinium | 90 Th Thorium | 91 Pa Protactinium | 92 U Uranium | 93 Np Neptunium | 94 Pu Plutonium | 95 Am Americium | 96 Cm Curium | 97 Bk Berkelium | 98 Cf Californium | 99 Es Einsteinium | 100 Fm Fermium | 101 Md Mendelevium | 102 No Nobelium | 103 Lr Lawrencium |

Bendall S.C. et al. *TRENDS in Immunology* (2012)

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.

Bendall SC et. al. (2012) Trends Immunol

Examples: Data Analysis/Display Methods



Important work:

Critical assessment of automated flow cytometry data analysis techniques

Nima Aghaeepour¹, Greg Finak², The FlowCAP Consortium³, The DREAM Consortium³, Holger Hoos⁴, Tim R Mosmann⁵, 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, ...

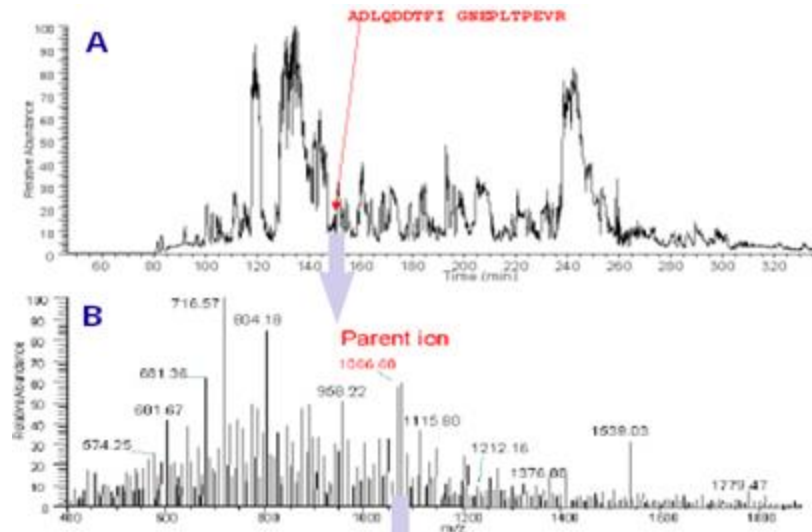
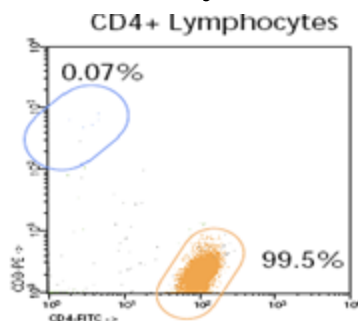
Measurement

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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