

# Cell and Organelle Purification for Proteomics

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Ben Verwer, **Diether Recktenwald** @BD Biosciences

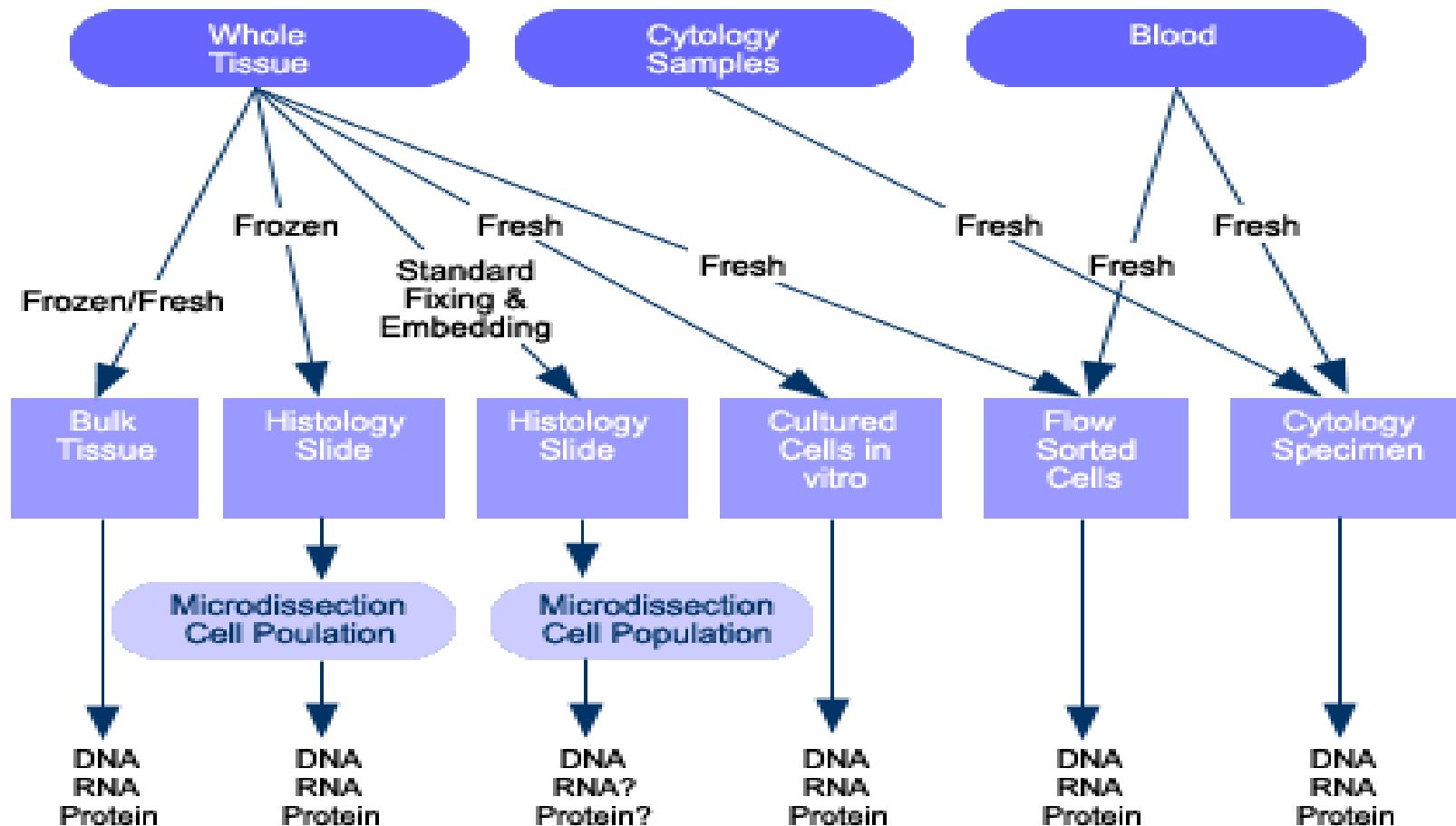
Contact email of presenter:  
[diether\\_recktenwald@bd.com](mailto:diether_recktenwald@bd.com) or [diether@att.net](mailto:diether@att.net)

**BD Biosciences**

Clontech  
Discovery Labware  
Immunocytometry Systems  
Pharmingen

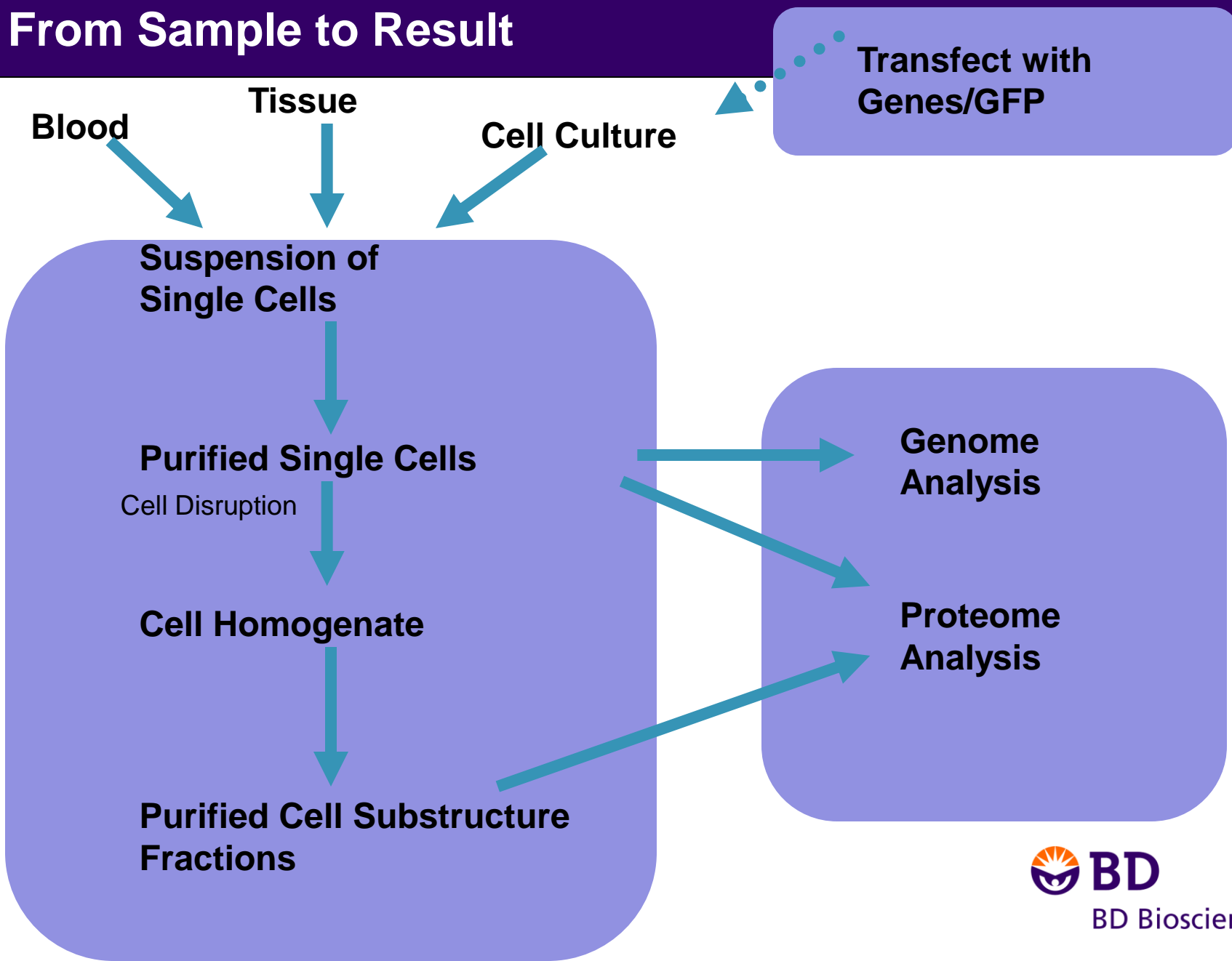


# Tissue Proteomics, Identifying Disease-related Proteins



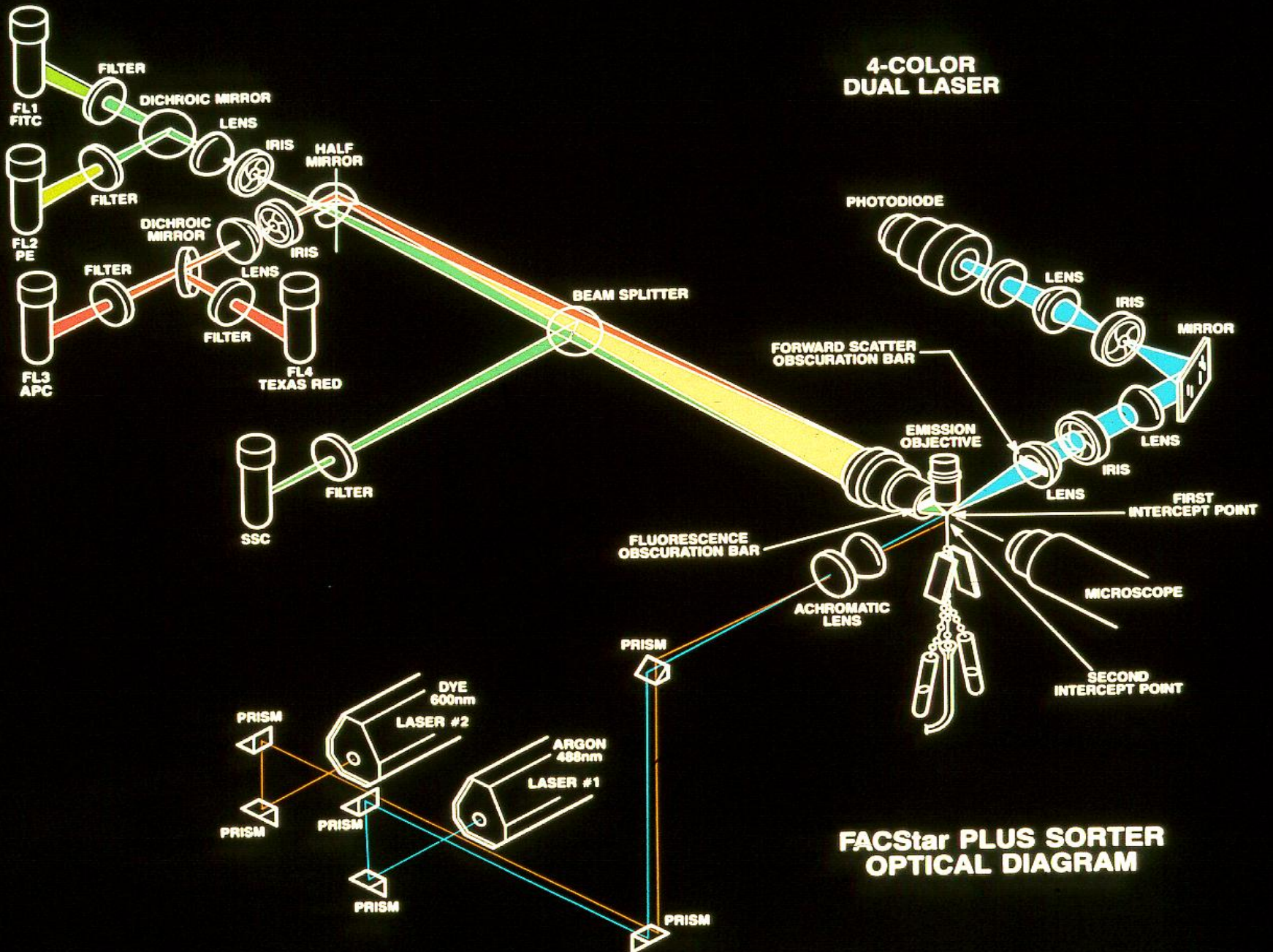
<http://cgap-mf.nih.gov/BtoB/BtoBSampleAcquisitionAndProcessing.html>

# Proteomics: From Sample to Result

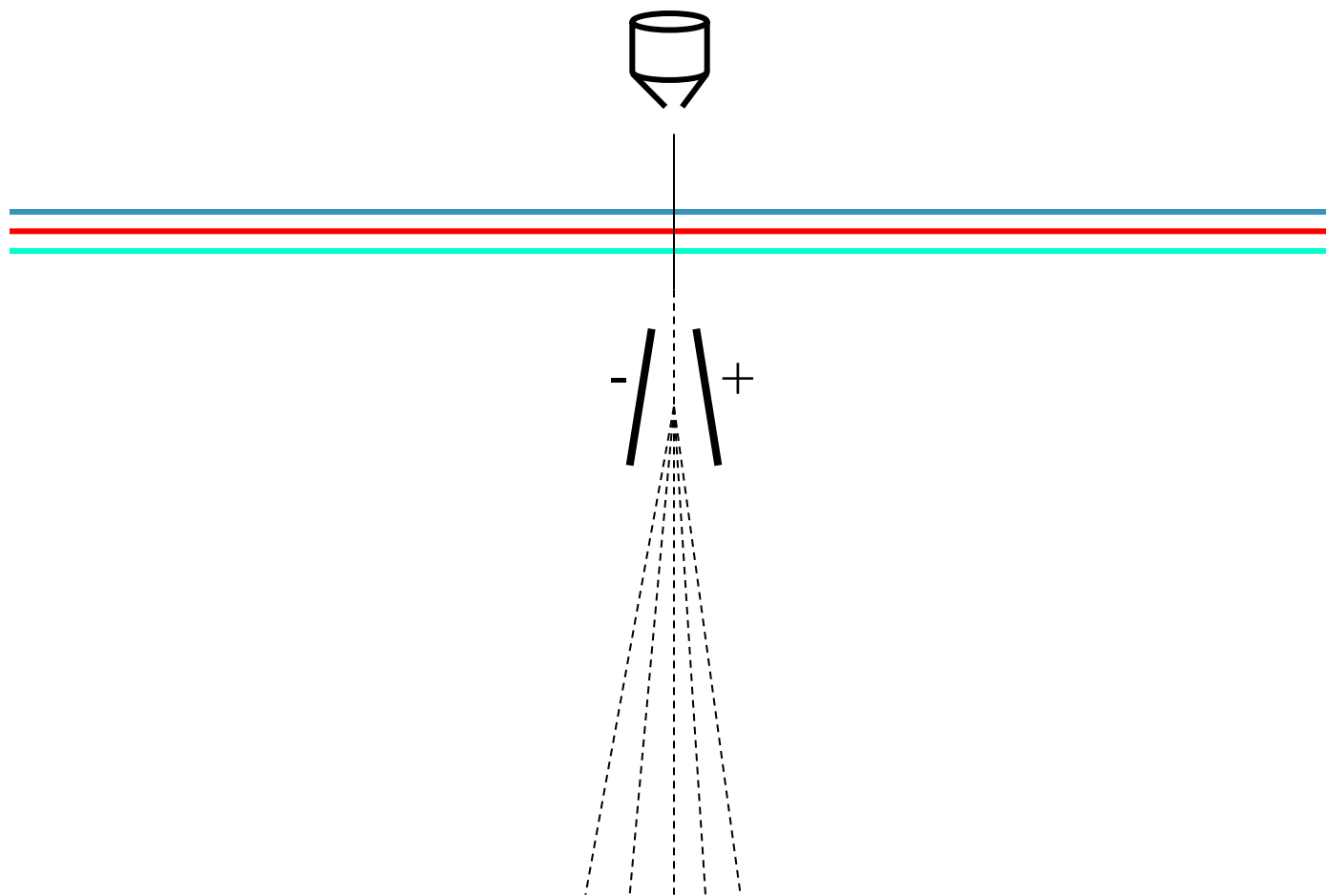


# FACS Cell Sorter

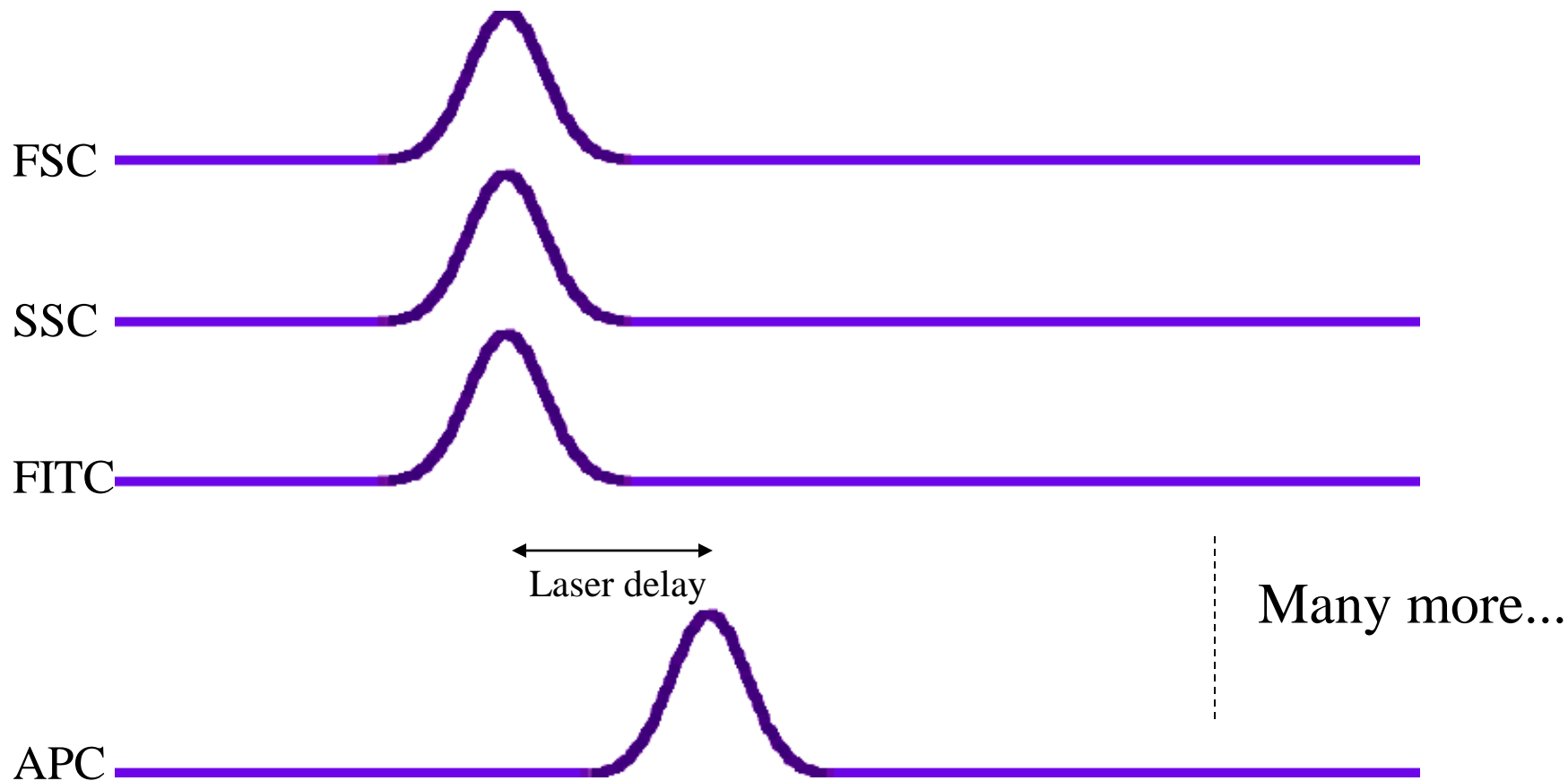




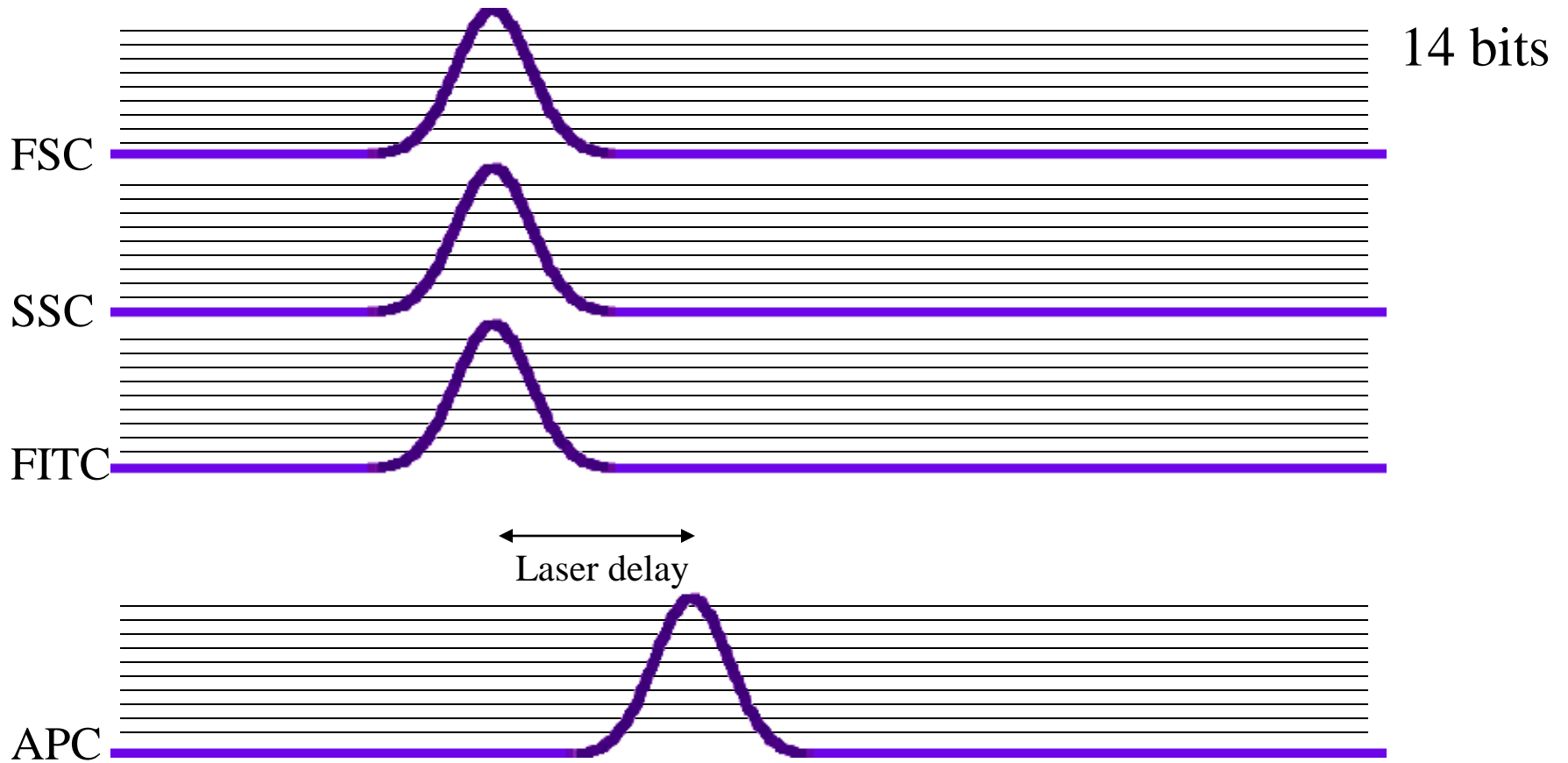
# FACS



# Analog Signals

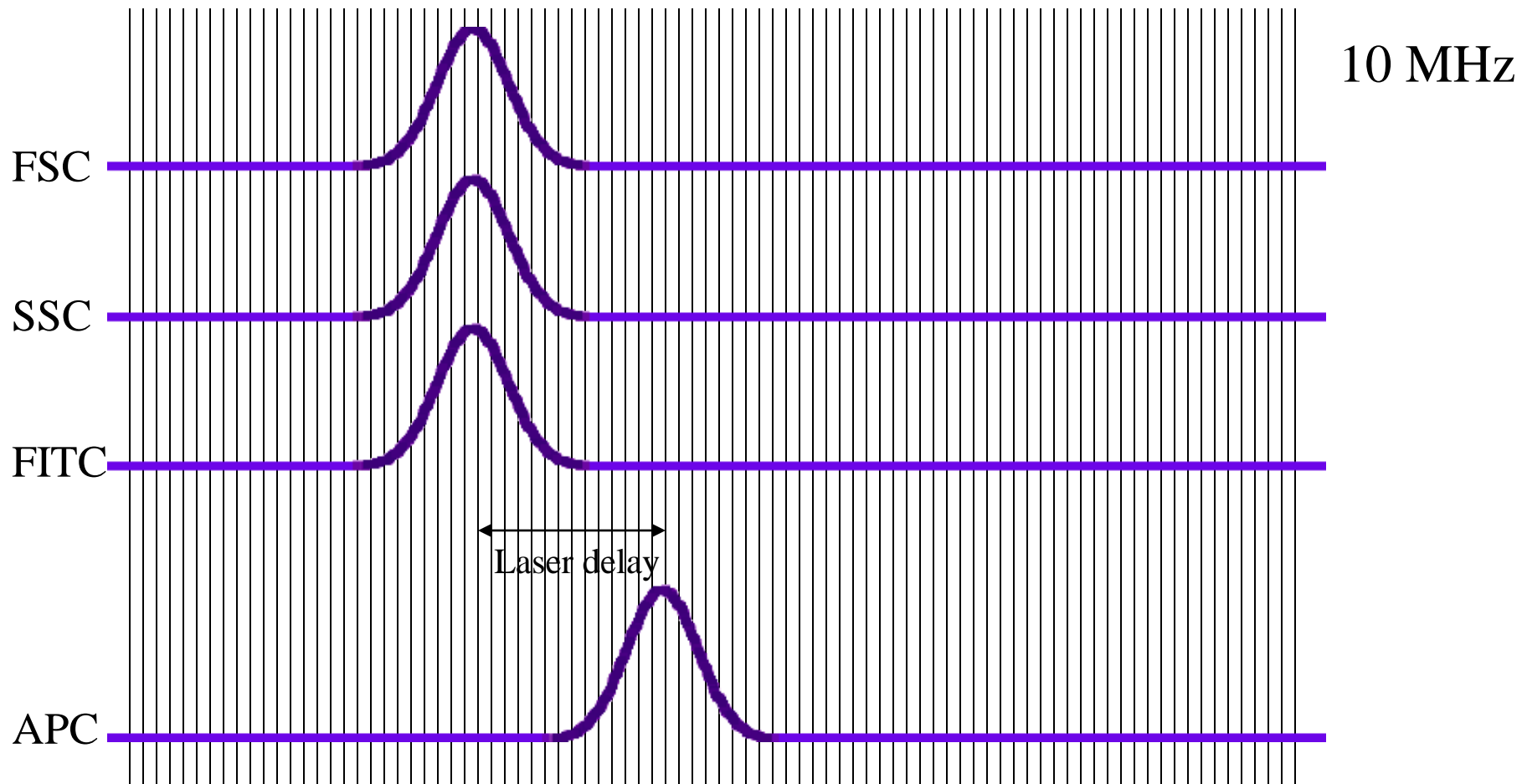


# Digitize in 16,384 Levels





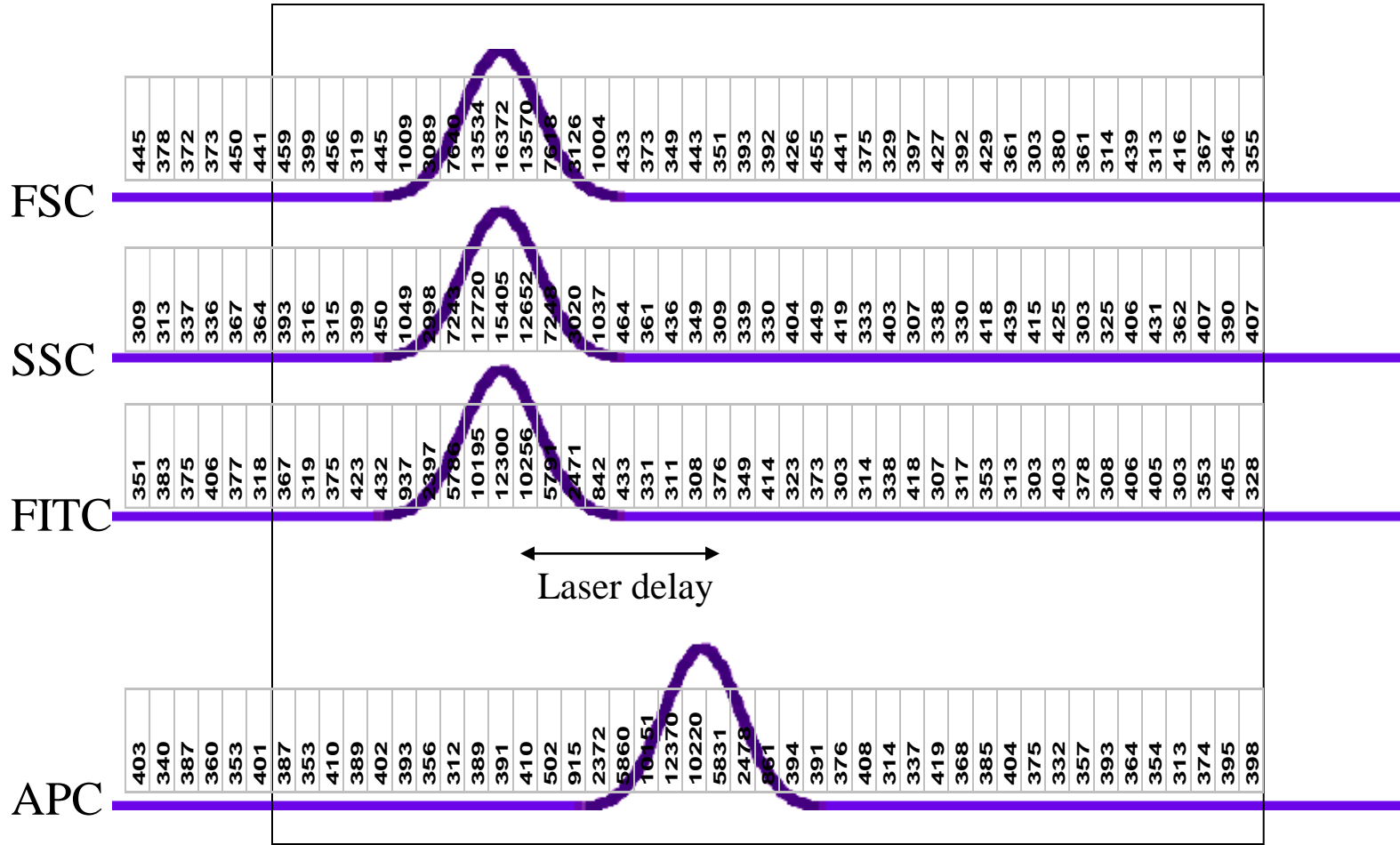
# Sample 10,000,000 per Second



# Digitization and Sampling

- Digitize in 14-bits
  - 16,384 levels
- Sample at 10Mhz
  - 10 million times/sec
- 16 channels

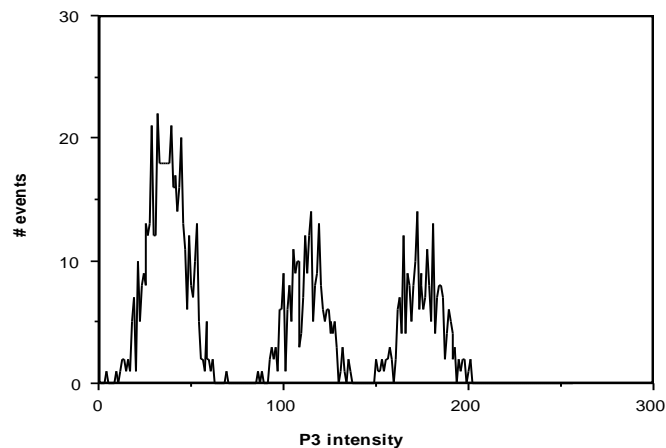
# Numbers in Memory



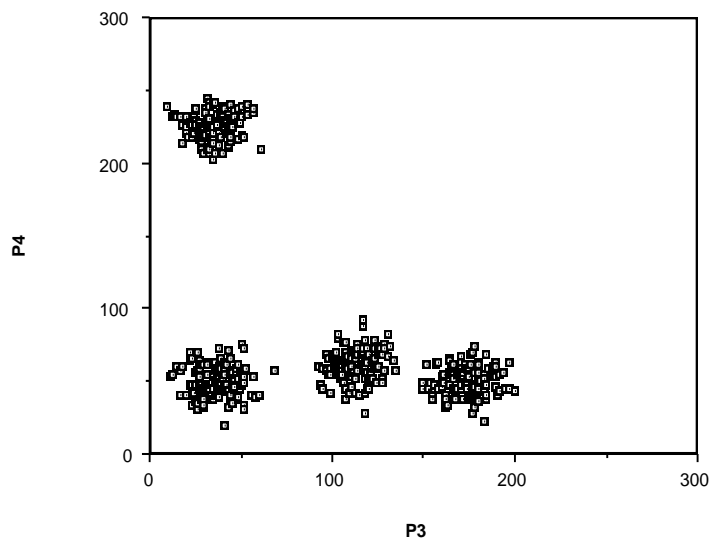
# Flow Cytometer Data

| 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    |

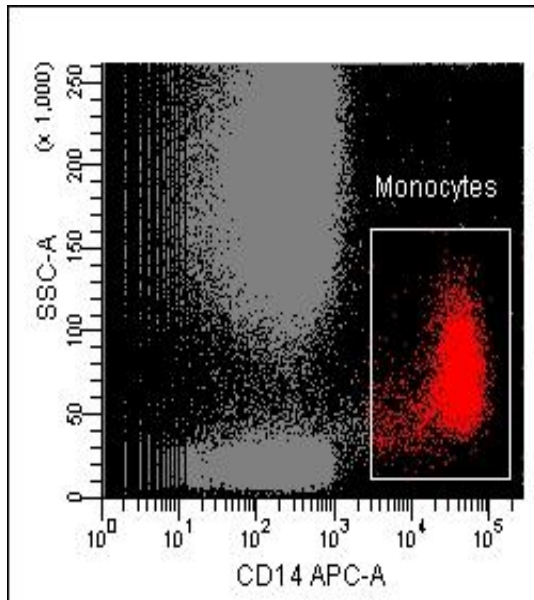
Event histogram



"Dotplot"

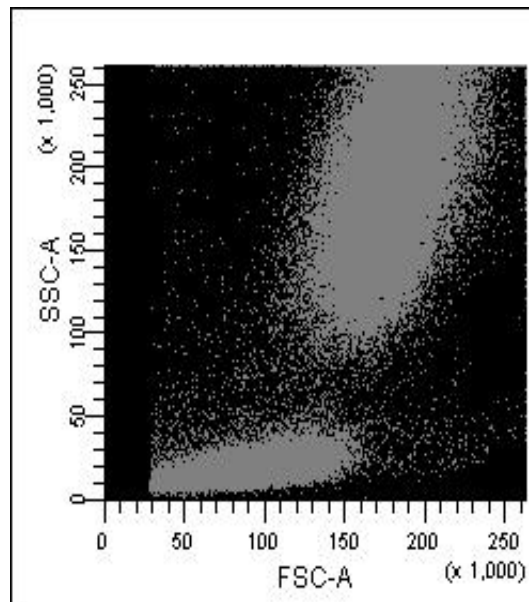
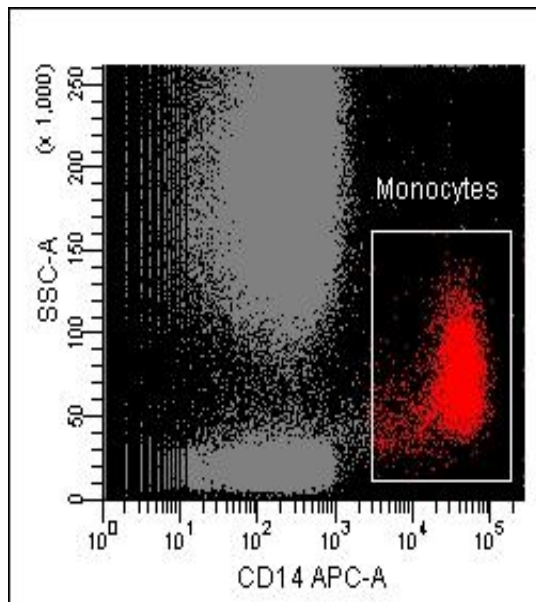


# Six color example

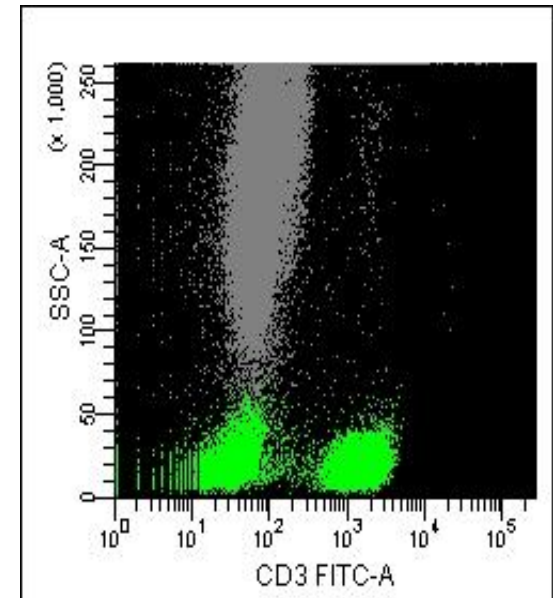
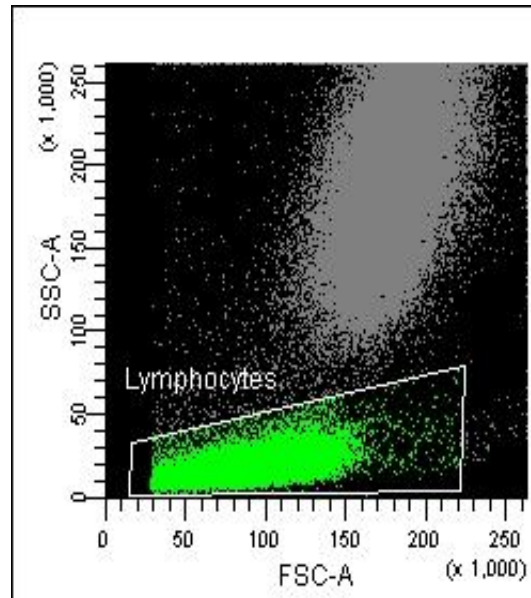
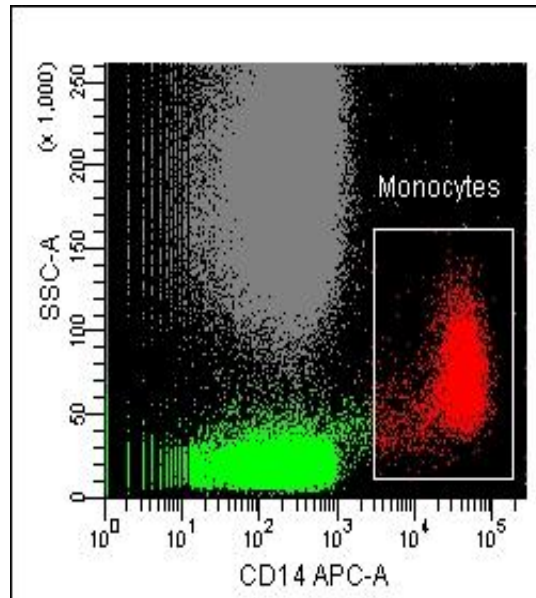


- CD3 FITC
- CD56 PE
- CD8 PE-Texas Red
- CD19 PE-Cy7
- CD14 APC
- CD4 APC-Cy7

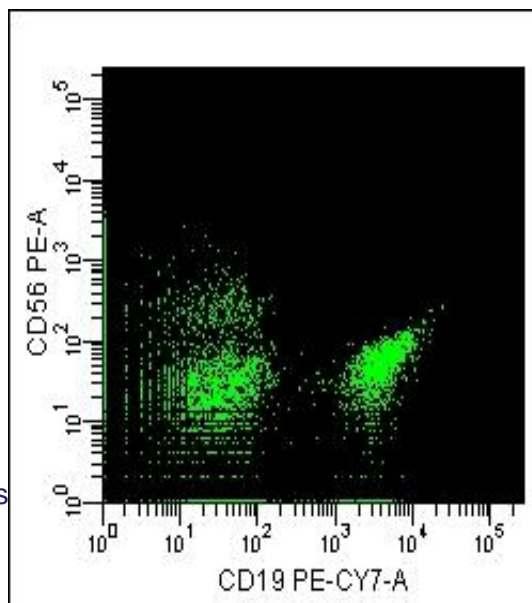
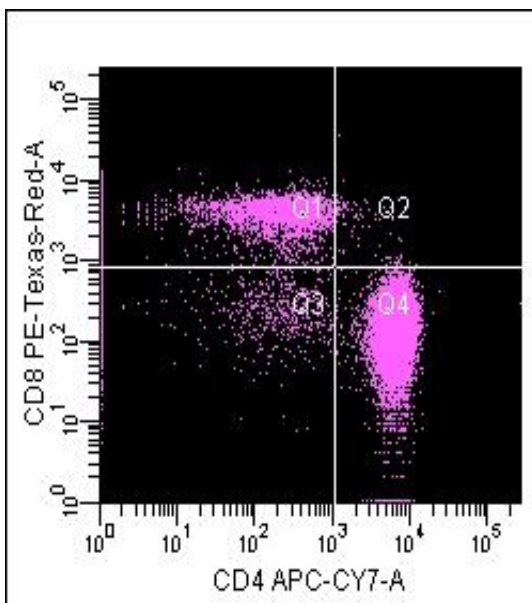
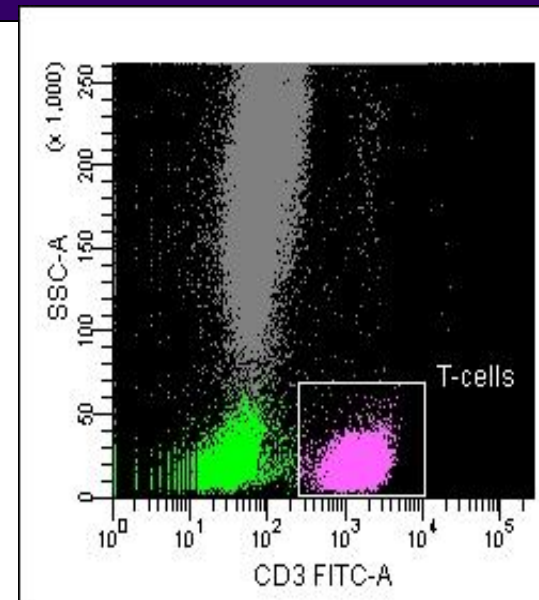
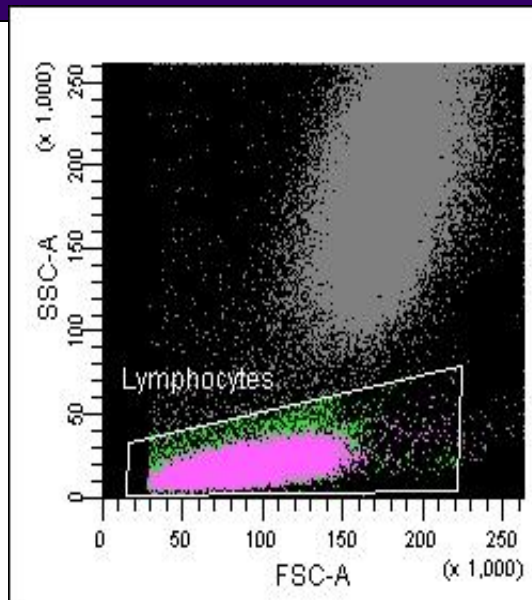
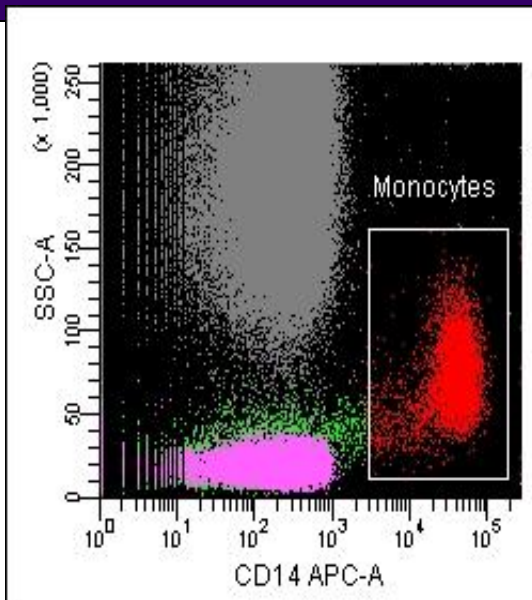
# Six color example



# Six color example



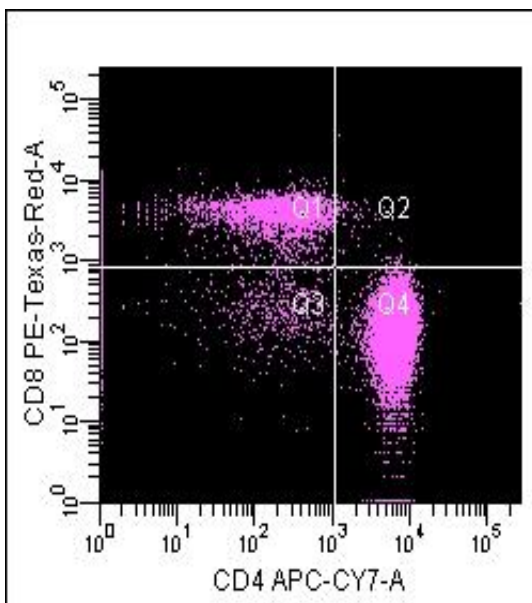
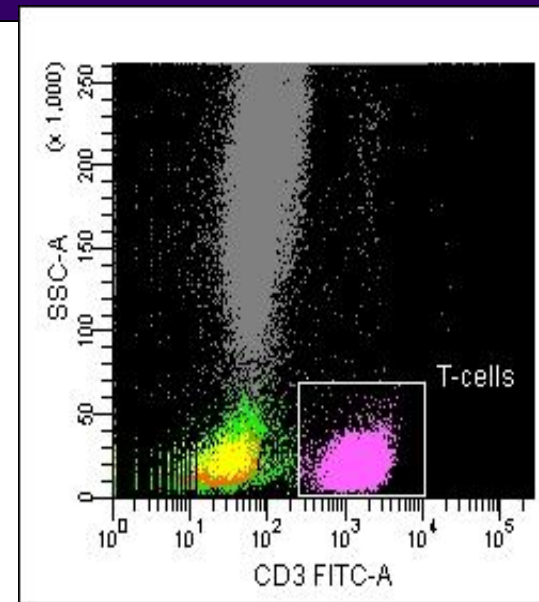
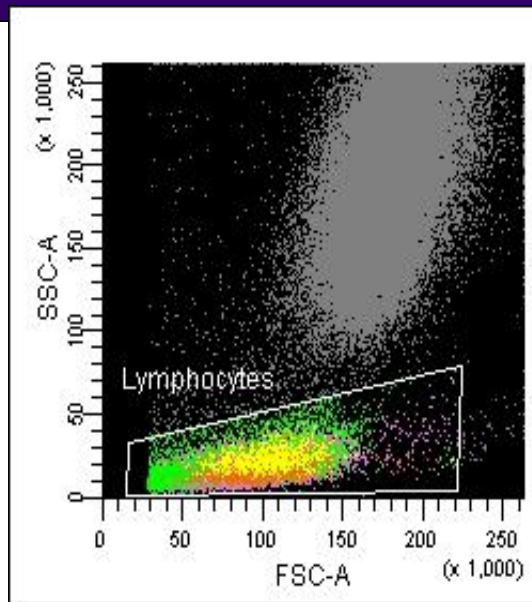
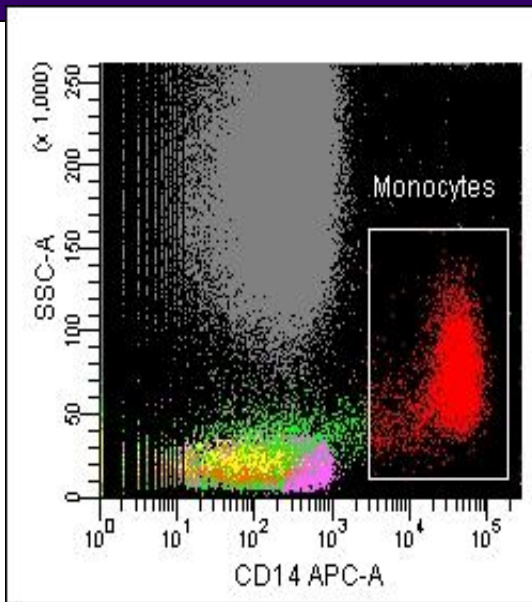
# Six color example



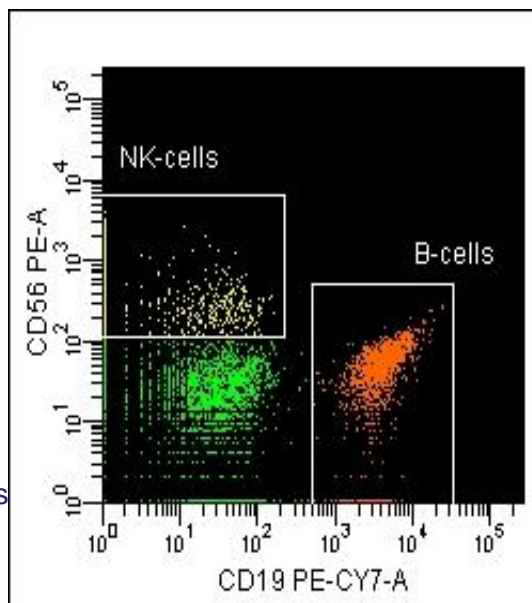
ems



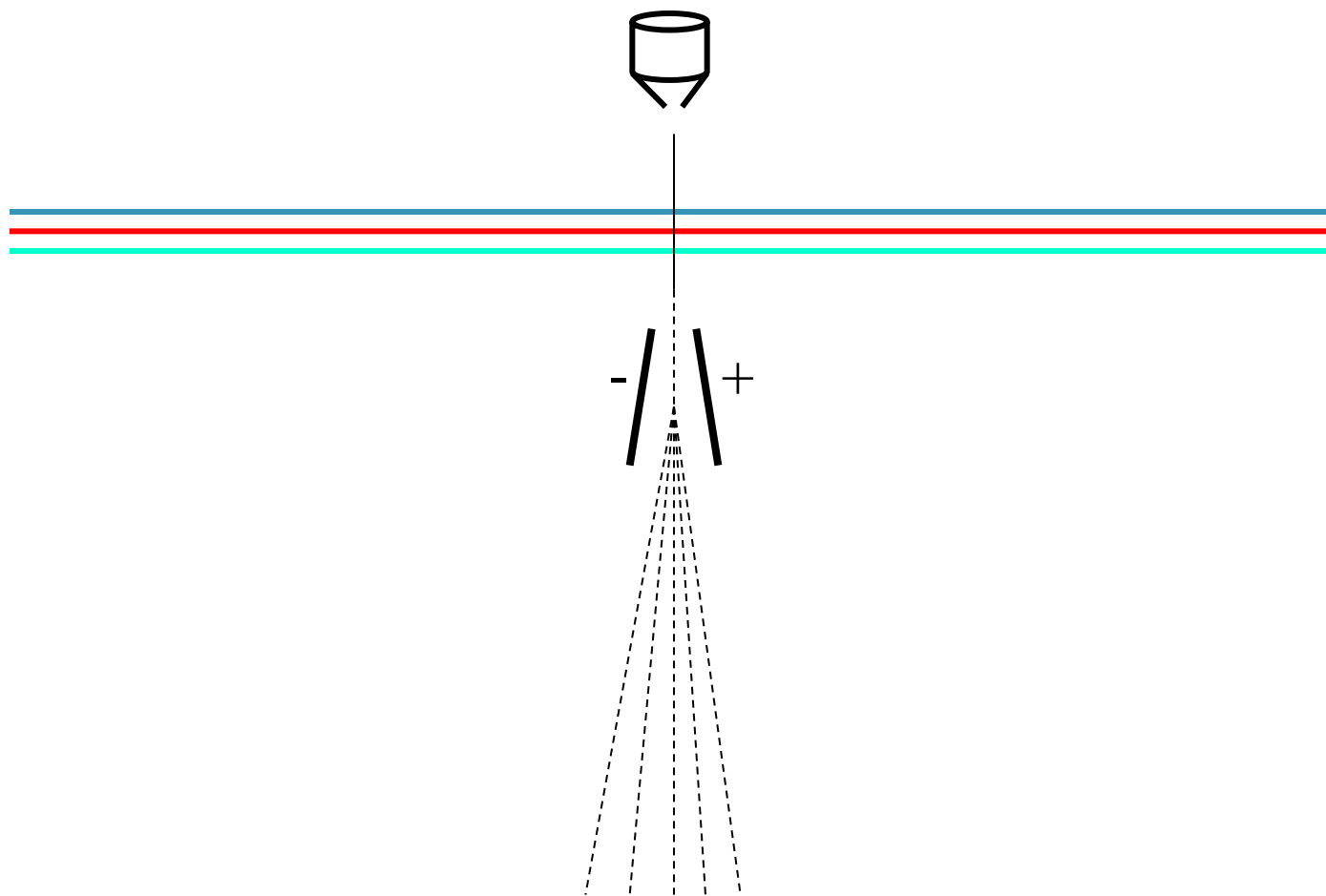
# Six color example



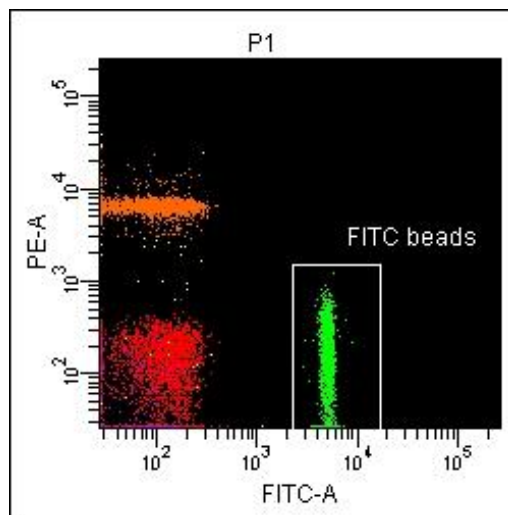
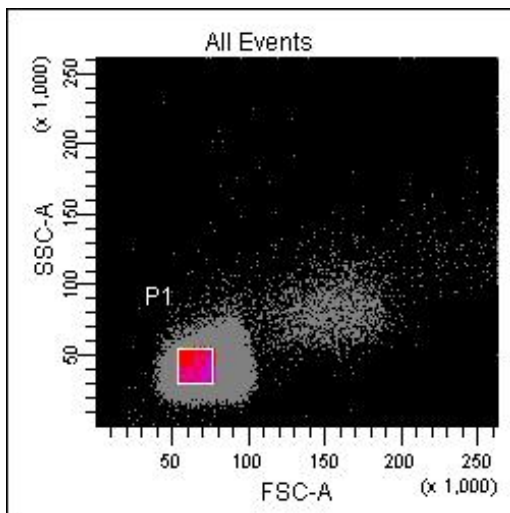
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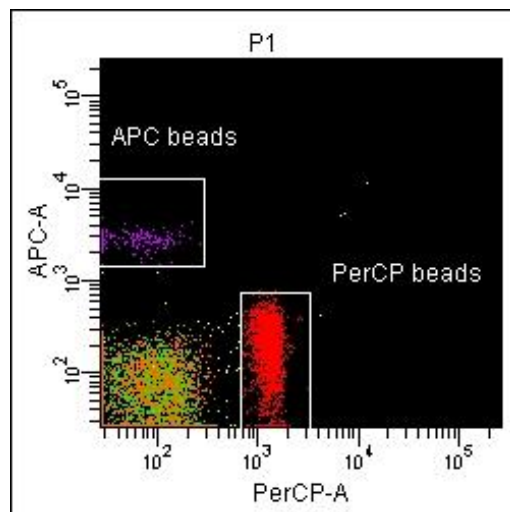
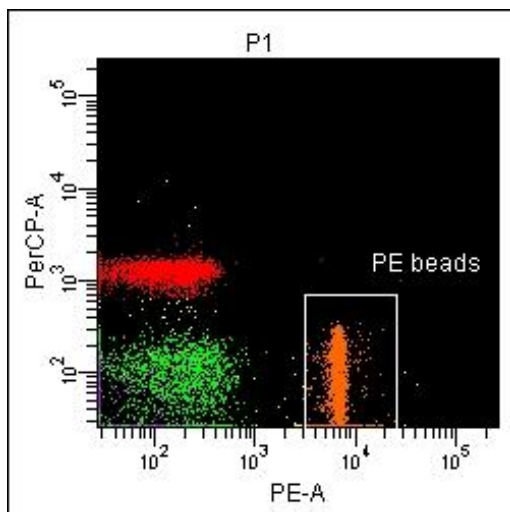
# FACS



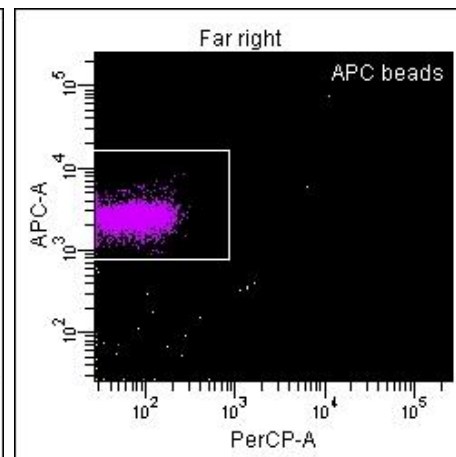
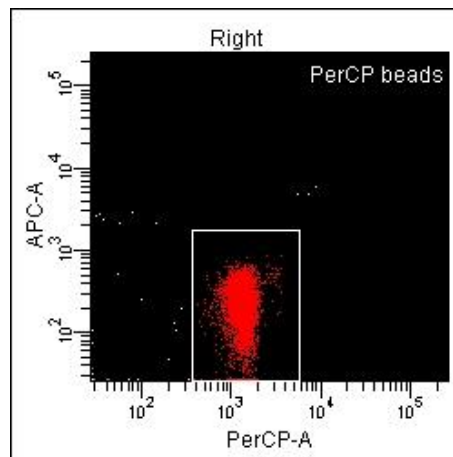
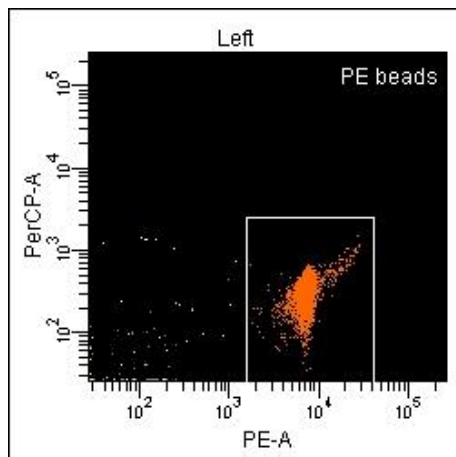
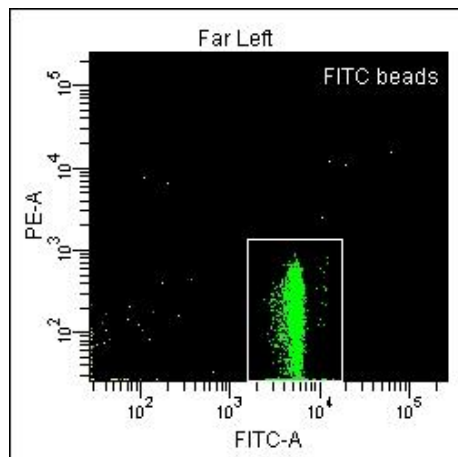
# Pre- Sort



45 psi  
64 kHz  
20,000 events/s



# Purity



FITC

8.2%

99.1%

PE

9.5%

99.2%

PerCP

8.0%

99.5%

APC

0.6%

99.4%

# Recovery, 60kHz, 6% population

| Event Rate                  | 4k/sec    | 20k/sec    | 24k/sec    |
|-----------------------------|-----------|------------|------------|
| DiVa sort counter           | 15k       | 46k        | 48k        |
| Analog sort counter         | 14k       | 38k        | 39k        |
| <b>More cells with DiVa</b> | <b>7%</b> | <b>23%</b> | <b>23%</b> |
| Theoretical recovery        | 90%       | 63%        | 58%        |
| Diva recovery               | 88%       | 65%        | 57%        |

# Molecule Copy Numbers

| <b>Molecule</b> | <b>Per T-cell</b>     | <b>fmoles/10<sup>6</sup> cells</b> | <b>LC-MS</b> |
|-----------------|-----------------------|------------------------------------|--------------|
| CD3             | 8.1 x 10 <sup>4</sup> | 130                                | ++           |
| CD4             | 5.9 x 10 <sup>4</sup> | 98                                 | -            |
| CD8             | 1.4 x 10 <sup>5</sup> | 230                                | +            |
| CD11a           | 2.7 x 10 <sup>4</sup> | 45                                 | +            |
| CD16            | 7.9 x 10 <sup>4</sup> | 130                                | +            |
| CD18            | 3.1 x 10 <sup>4</sup> | 52                                 | +            |
| CD45            | 1.9 x 10 <sup>5</sup> | 320                                | -            |

Appendix A, Cell Separation Methods and Applications. Marcel Dekker 1998.  
Recktenwald D and Radbruch A, eds.

# Parameters for Selection of Cell Subsets

Analyse and Sort based on:

- light scatter
- immunofluorescence
- fluorescent in-situ hybridization
- DNA content
- transfection with fluorescent proteins
- protein content
- auto-fluorescence
- enzyme activity
- pH
- redox potential
- other components detectable by fluorescence

# DNA Content as Tumor Marker

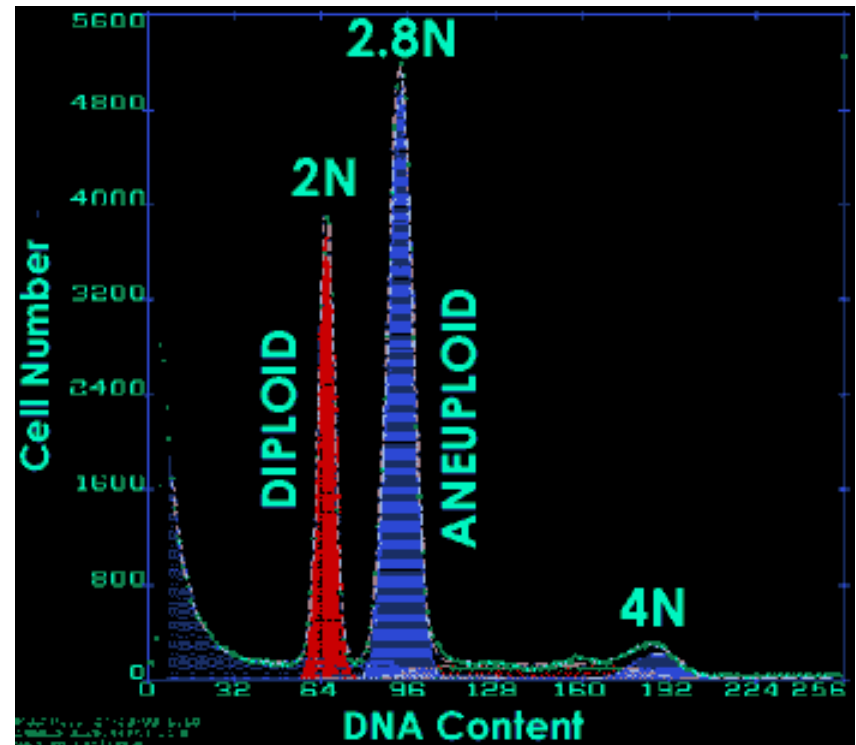
## Advantages of FACS™ based sorting

- Yield large numbers of purified, primary cells (i.e., not cultured) from patients.
- Yield high quality biomolecules.
- May potentially provide molecular profiles very similar to cells in vivo due to their rapid processing after removal from the patient.
- Can be separated into specific subsets of cells based on molecular markers on the cell type of interest.

<http://cgap-mf.nih.gov/BtoB/BtoBSampleAcquisitionAndProcessing.html>

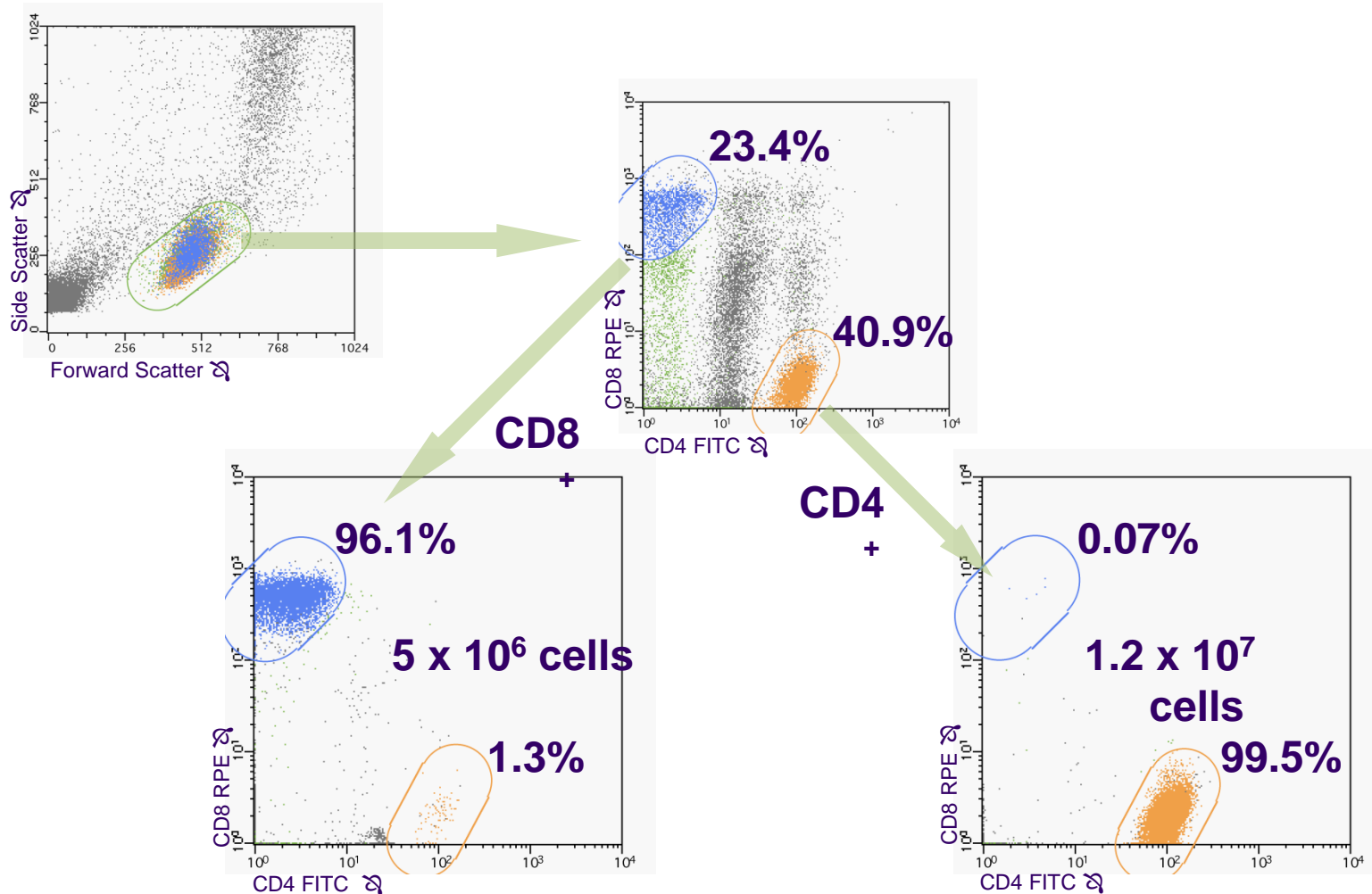
In Barrett's esophagus, flow cytometry can be used to identify patients who are at low- or high-risk for progression to high grade dysplasia or cancer.

([www.barrettsinfo.com/content/6a1\\_use\\_of\\_flow\\_cytometry](http://www.barrettsinfo.com/content/6a1_use_of_flow_cytometry))





# Analysis of Sorted Populations



# Cell-surface Proteins Identified On Sorted T-cells

## CD4+ Cells

|   |
|---|
| T-cell receptor beta chain VJ region ( <b>CD3</b> )                             |
| Integrin alpha L precursor; antigen <b>CD11A</b> (p180),                        |
| Integrin alpha 9 protein  |
| Integrin alpha-7B   |
| Low affinity IgG FC region receptor III-A precursor ( <b>CD16-A</b> )           |
| Complement receptor type I (C3B/C4B receptor) ( <b>CD35</b> antigen)            |
| T-cell surface glycoprotein E2 ( <b>CD99</b> )                                  |
| Mast/stem cell growth factor receptor precursor (C-KIT) ( <b>CD117</b> antigen) |
| Interleukin-1 receptor, type II precursor (Antigen <b>CDW121B</b> )             |
| Interleukin-8 receptor type B ( <b>CDW128B</b> )                                |
| Interleukin-2 receptor gamma chain ( <b>CD132</b> )                             |

## CD8+ Cells

|  |
|--|
| T-cell antigen receptor alpha chain ( <b>CDR3</b> )                        |
| T-cell surface glycoprotein <b>CD8</b> beta chain isoform                  |
| Fc-gamma receptor III-2 ( <b>CD 16</b> )                                   |
| Leukocyte adhesion protein beta chain ( <b>CD18</b> ) precursor            |
| Leukocyte surface antigen <b>CD47</b> precursor                            |
| Integrin, alpha-2 ( <b>CD49B</b> ; alpha-2 subunit of VLA-2 receptor)      |
| Integrin, alpha V (vitronectin receptor, antigen <b>CD51</b> )             |
| Interleukin-22 receptor  |
| Transferrin receptor (p90, <b>CD71</b> )                                   |
| Putative. B7,3 molecule of <b>CD80-CD86</b> family                         |
| Leukocyte differentiation antigen <b>CD84</b>                              |
| Cell-surface antigen heavy chain (4F2HC) ( <b>CD98 ANTIGEN</b> )           |
| T-cell surface glycoprotein E2 ( <b>CD99</b> )                             |
| Tumor necrosis factor receptor 2 precursor (TBPII) (P80) ( <b>CD120B</b> ) |
| Protocadherin beta 9 precursor ( <b>CDB9</b> )                             |

# Cell Isolation and Purification from Tissues for Proteomics

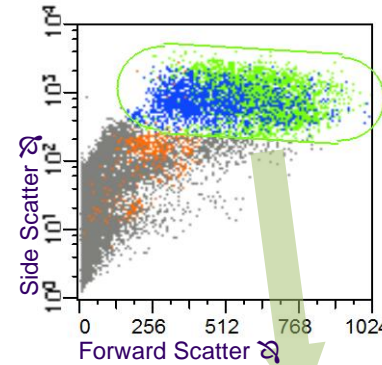
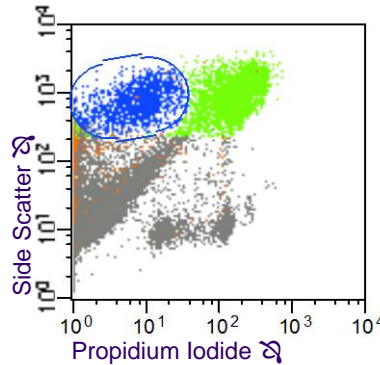
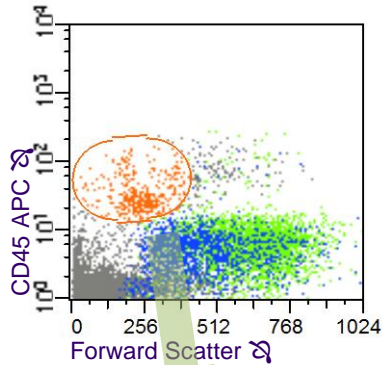
- Mechanical or Enzymatic Tissue Disruption
- FACS™ or immunomagnetic sorting for the purification of cell subsets
- Protein extraction
- Analysis by LC-MS, 2D gels or protein micro-arrays

(MS analysis by ThermoFinnigan demonstrated, that enough low abundant protein can be obtained for global MS Id.)

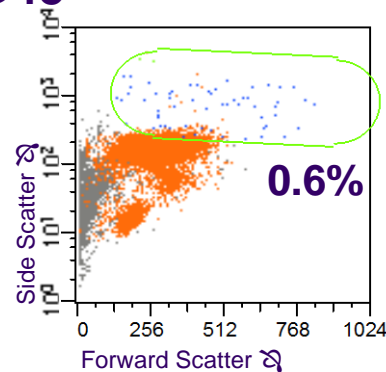
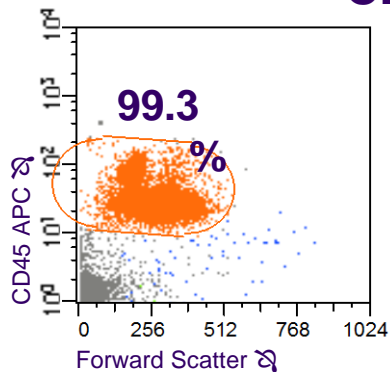
# Tissue Proteomics, Liver Model

- Collagenase perfusate of human liver (BD Gentest, Woburn, MA)
- Anti-CD45-APC / propidium iodide  $\otimes$  Sort (viability, CD45 expression, scatter)  $\otimes$  LC-MS/MS analysis

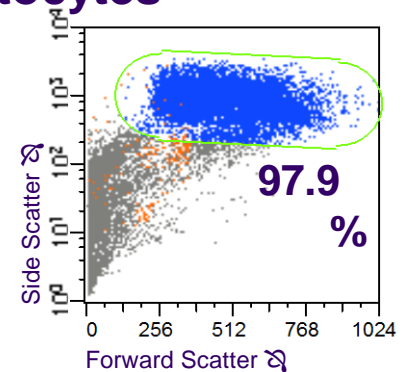
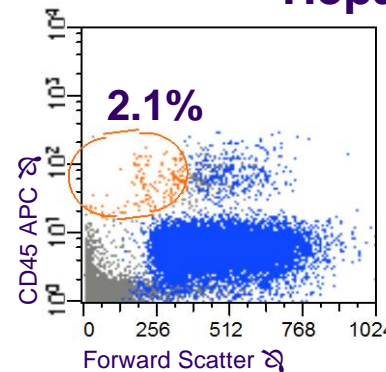
Starting cell suspension



CD45<sup>+</sup>



Hepatocytes



# Preliminary Results - Hepatocyte Fraction

- 35 proteins identified with high confidence from 54 unique peptides
- 8 mitochondrial-specific proteins and precursor proteins
- Hepatic arginase
- Large proportion of cytoplasmic proteins

# Organelle Purification for Proteomics

## Identification of Specific Organelles for FACS™

- Specific Enzymes with Fluorogenic Substrates
- Component-Specific Dyes
- Autofluorescence
- Organelle Targeted Vectors for Expression of Fluorescent Proteins

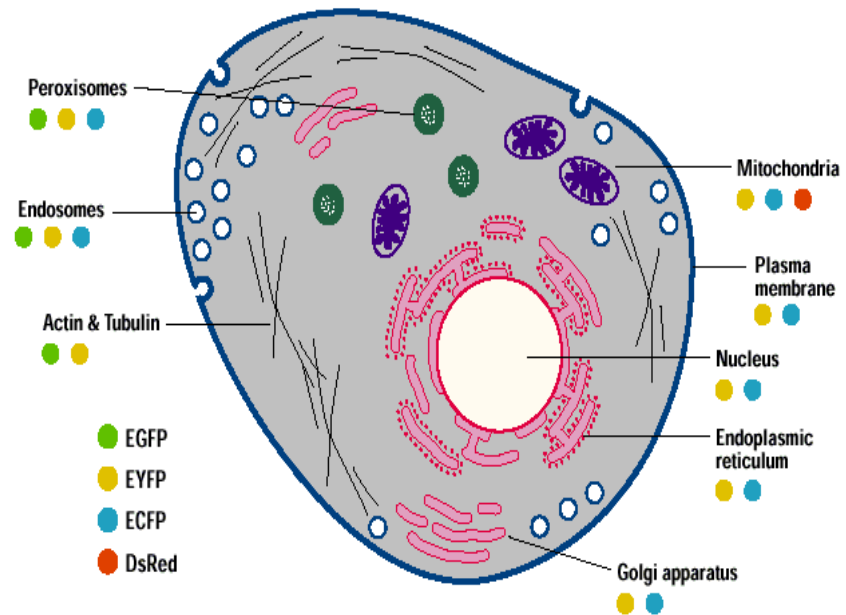
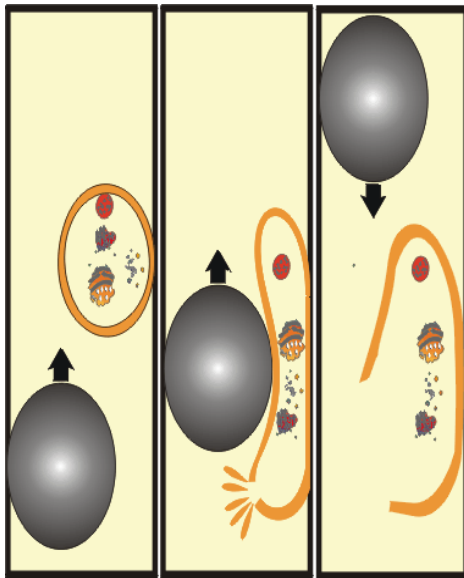


Figure 9. Organelles targeted by Living Colors™ Subcellular Localization Vectors.

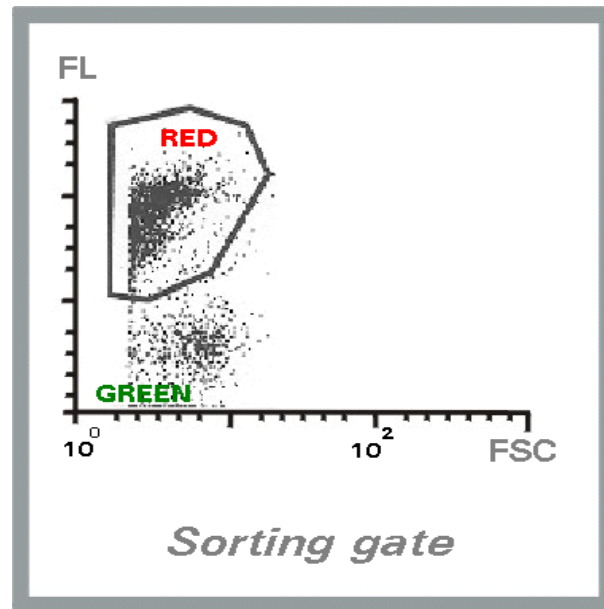
# Organelle Purification for Proteomics

- Cell disruption to release sub-cellular structures (organelles)
- Purification with anti-body coated Imag magnetic particles and cell sorting based on antibody reactivity or other specific properties.

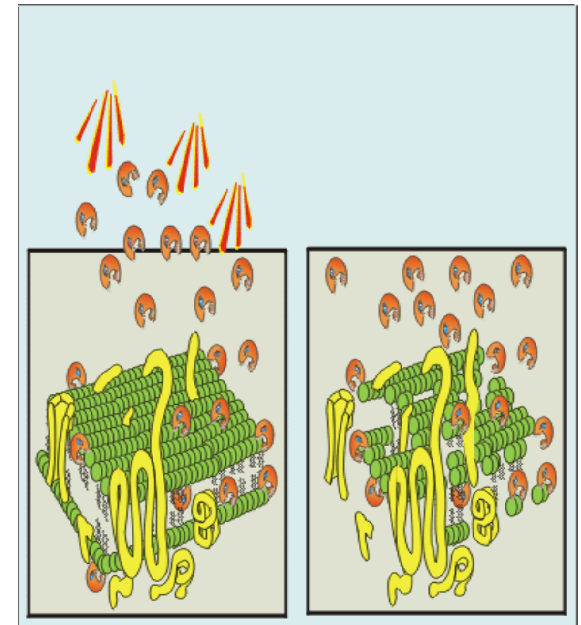
From the website of the Catholic University of Nijmegen



Cell breakage



FACS organelle sorting



Protein extraction

# Mitochondria

Homogenate  
from mouse liver  
cells



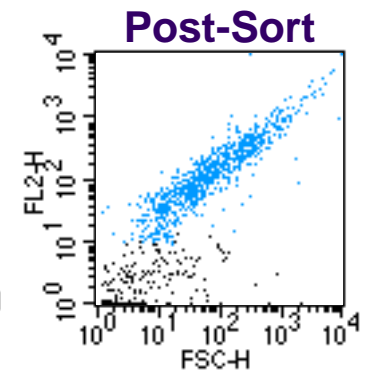
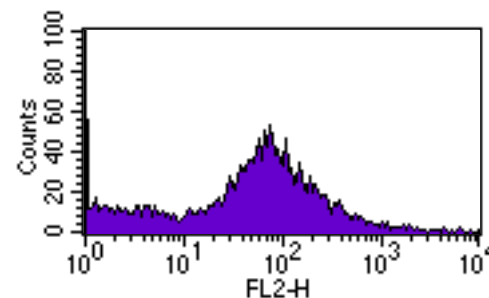
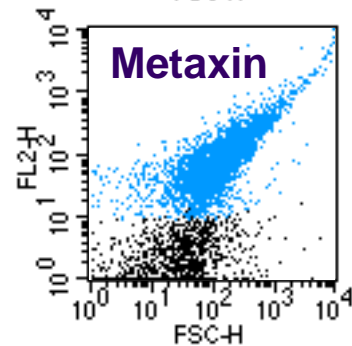
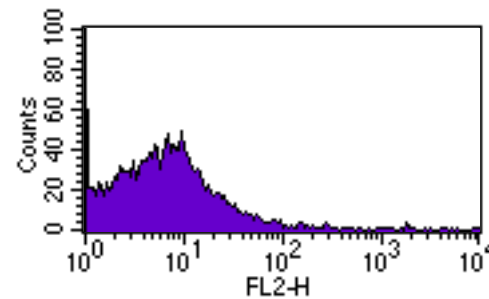
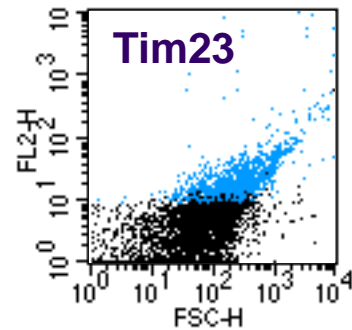
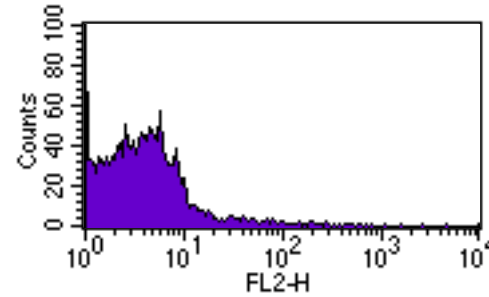
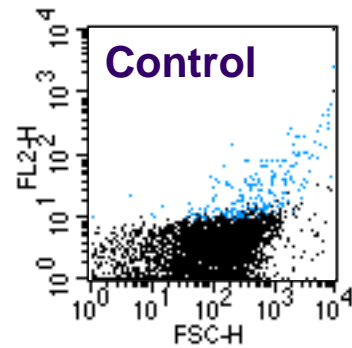
Antibodies  
against metaxin  
or Tim23 (BD  
Biosciences  
Pharmingen)



Anti-Kappa-RPE



Analysis &  
sorting

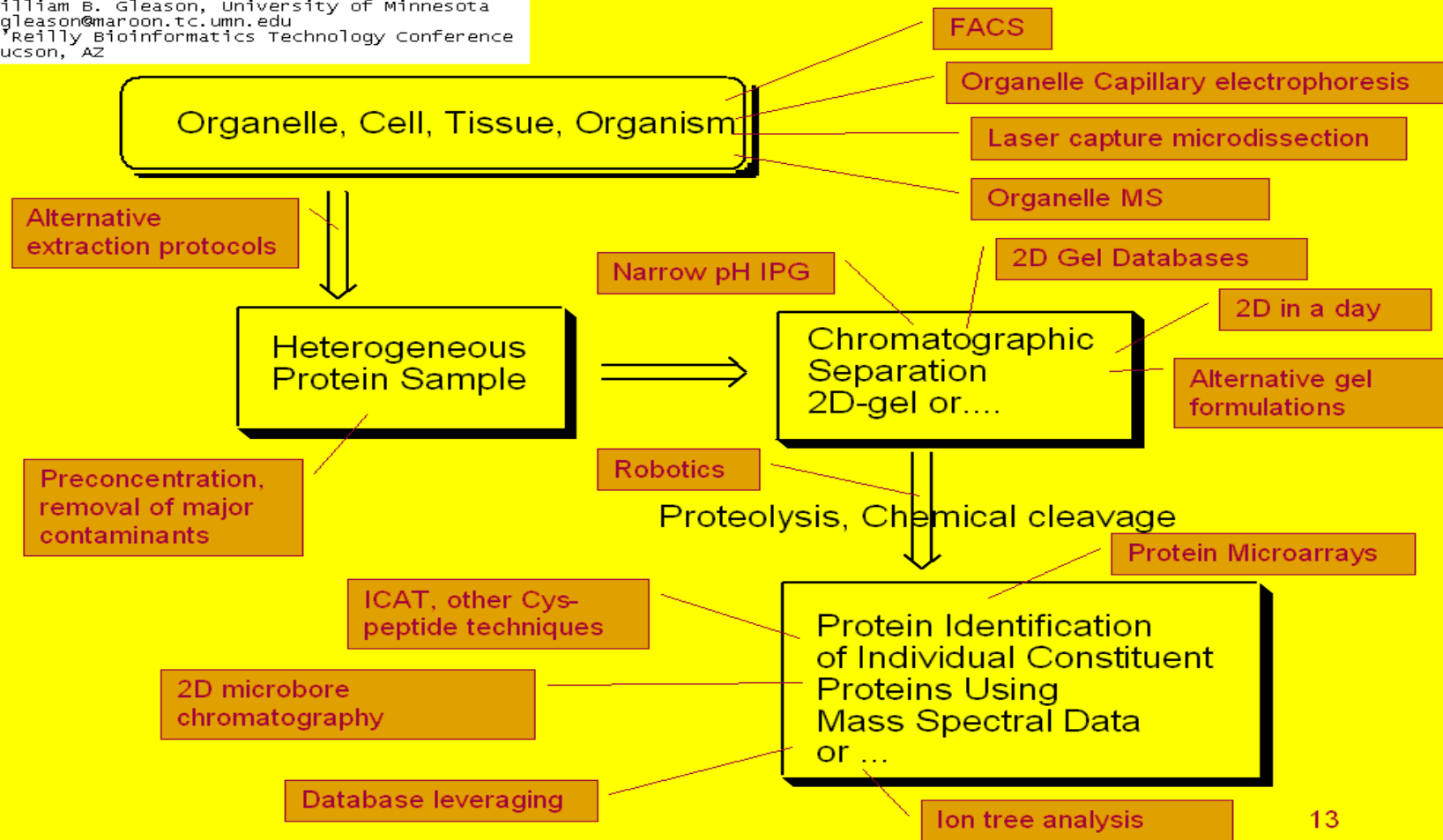




# Organelle/Cell/Tissue Proteomics

## Summary

William B. Gleason, University of Minnesota  
 bgleason@maroon.tc.umn.edu  
 O'Reilly Bioinformatics Technology Conference  
 Tucson, AZ



# Conclusions

- Flow cytometric and immuno-magnetic particle separation methods yield sufficient highly purified preparations for proteomic analysis of low abundance proteins.
- Flow cytometry offers the best flexibility in parameter selection for cell and organelle separations, while immunomagnetic techniques provide higher throughput.

**END**