INSTITUT PASTEUR — UNIVERSITE PIERRE & MARIE CURIE

**The 2nd PSU International Teaching Platform on Tumour Immunology and Immunotherapy**

Jointly organized by
Prince of Songkla University, Université Pierre et Marie Curie (Paris 6) and Institut Pasteur

December 15 – 20, 2003
At The Department of Biomedical Sciences
Faculty of Medicine, Prince of Songkla University,
Hat Yai, Songkhla, Thailand

Lecture 11:
Flow cytometry and intracellular cytokine detection
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# Few notions in flow cytometry and intracellular cytokine detection

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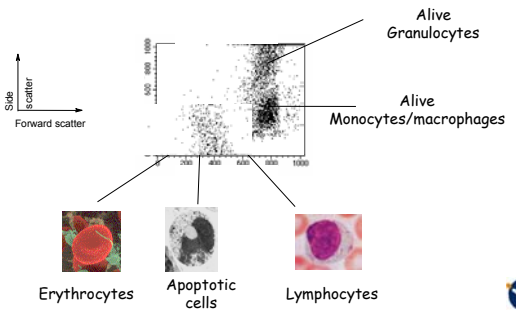
## Detection of the blood subsets using SSC/FCS parameters

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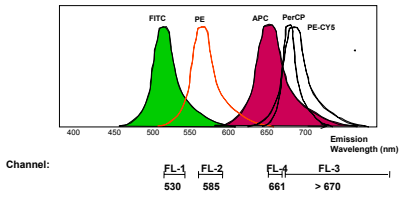
## Usual dye coupled with antibodies




Channel:

| FL-1 | FL-2 | FL-4 | FL-3 |
|---|---|---|---|
| 530 | 585 | 661 | > 670 |

**FITC: Fluorescein Isothiocyanate**
**PE: Phycoerythrin**
**APC: Allophycocyanin (*)**
**perCP: Peridinin Chlorophyll Protein**
**PE-Cy5: PE + Cyanine-5**

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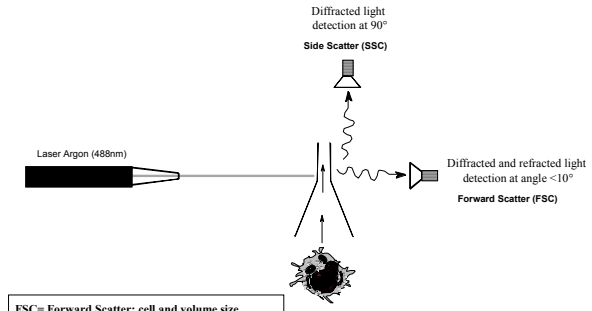
## Size (FCS)-granularity (SSC) detection




**FSC= Forward Scatter: cell and volume size**
**SSC= Side scatter: cell complexity and granularity**

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## Subsets detectable by flow cytometry

| | Human | Mouse |
|---|---|---|
| - Leukocytes: | CD45 | CD45 (2 alleles: Ly5.1, Ly5.2) |
| - T Lymphocytes: | CD2, CD3, CD4, CD8, $\alpha\beta$, $\gamma\delta$ (different chains) | |
| - NK: | CD16, CD56, KIR | CD56, NK1.1, KIR |
| - B Lymphocytes: | CD19, CD20 | |
| - Monocytes / macrophages: | CD4, CD44 | CD11b, CD14 |
| - Granulocytes: | CD15 | Anti-GR1 |
| - DC: | CD11c | |

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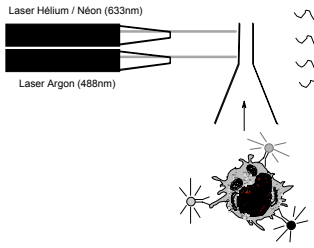
## Detection of activation/differentiation by cytometry

| | Human | Mouse |
|---|---|---|
| Naïve/Memory | CD45RA,CD45RO, CD62-L, CCR7 | CD45RB,CD44, CD62-L |
| Resting/Effector | CD69, CD25, HLA-DR | CD69, CD25 |



## Fluorescence detection




Detection of wavelength emitted by different excited dies

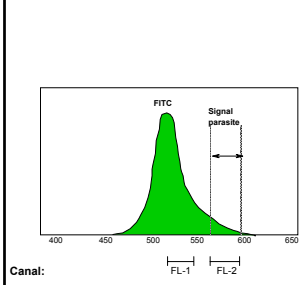


## Cytokines/Cytokine receptors detected by flow cytometry

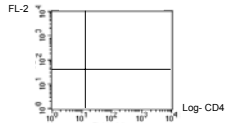
| | Human | Mouse |
|---|---|---|
| Cytokines | IL-1, IL-2, IL-4, IL-6, IL-8, IL-13, TNFα, γIFN | IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, GM-CSF, γIFN, TNFα |
| Cytokine receptors | G-CSF-R, GM-CSF-R, IL-2R, IL-3R, IL-5R, γc, IL-10R, IL-12R, | IL-4Rα, IL-3R, IL-6R IL-7Rα, IL-12Rβ, γ IFN, TNF-R, IL2R, γc, IL-15R, IL-10R |



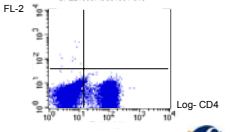
## Settings: the compensation problem




CD4 FITC staining not compensated in FL-2



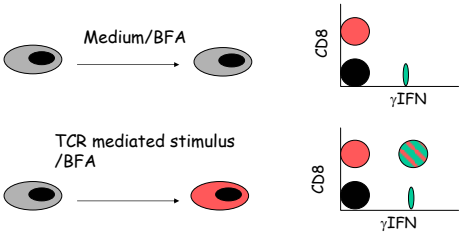

CD4 FITC staining compensated in FL-2






## Intracellular staining procedures 1

4-ON culture step:
cytokine accumulation for further detection






## Intracellular staining procedures 2