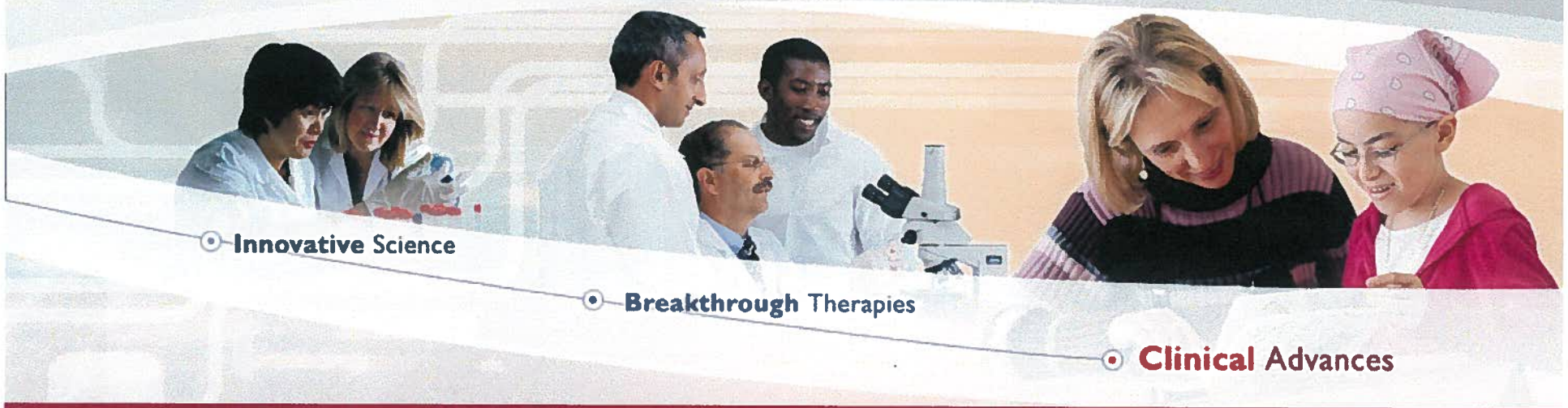
CENTER FOR CANCER RESEARCH

Connecting the Cancer Community



FLOW CYTOMETRY

16 November 2009

NATIONAL INSTITUTES OF HEALTH

NATIONAL CANCER INSTITUTE

37

Definitions

Flow Cytometry

A flow cytometer is a laser based instrument that measures properties of cells in single cell suspension in flow based on their fluorescence (extrinsic) and light scattering (intrinsic)

Flow Sorting

Sorting (separating) cells based on properties measured in flow

Also called **Fluorescence-Activated Cell Sorting (FACS)**

Advantages

Ability to look at individual cells

Ability to make rapid (10,000 cells/second) quantitative measurements on single cells

Ability to make multiple, simultaneous, correlated measurements per cell (multiparameter flow cytometry)

Ability to look for rare events (1 in 100,000)

Ability to isolate cells using these measurements at 20,000 cells/second

Disadvantages

Cells must be in single cell suspension

Can NOT measure cell morphology or ultrastructure or cell-cell relationship

Can NOT measure cells within a tissue

Does not provide images

Applications

DNA cell cycle / tumor ploidy

Immune cell subsets / AIDS monitoring

Hematology / leukemia immunophenotyping / differentiation therapies

Transplantation biology

Human and animal fertility

Chromosome analysis & sorting / Human Genome Project

Pharmacology, toxicology, microbiology, parasitology, marine biology, plant science, water testing

Cellular Properties measured by flow cytometry

Total DNA/cell, DNA synthesis, tumor ploidy, cell division

Apoptosis - membrane permeability, caspase, mitochondria, TUNEL, sub G0/G1 population

Any antigen (cell surface, cytoplasm, nucleus, organelle) that can be labeled with a fluorescent antibody - immunophenotyping, intracellular cytokines, phosphoproteins

Reporter molecules-GFP, YFP, CFP, red fluorescent proteins

Transport /efflux of fluorescent substrates

Intracellular calcium (kinetics)

Fluorescence

- **Fluorescence – form of light emitted by atoms or molecules when electrons fall from excited energy levels to their resting ground state**

- **Fluorochrome – a dye that absorbs light at a specific wavelength and then emits light of a different color at a longer wavelength**

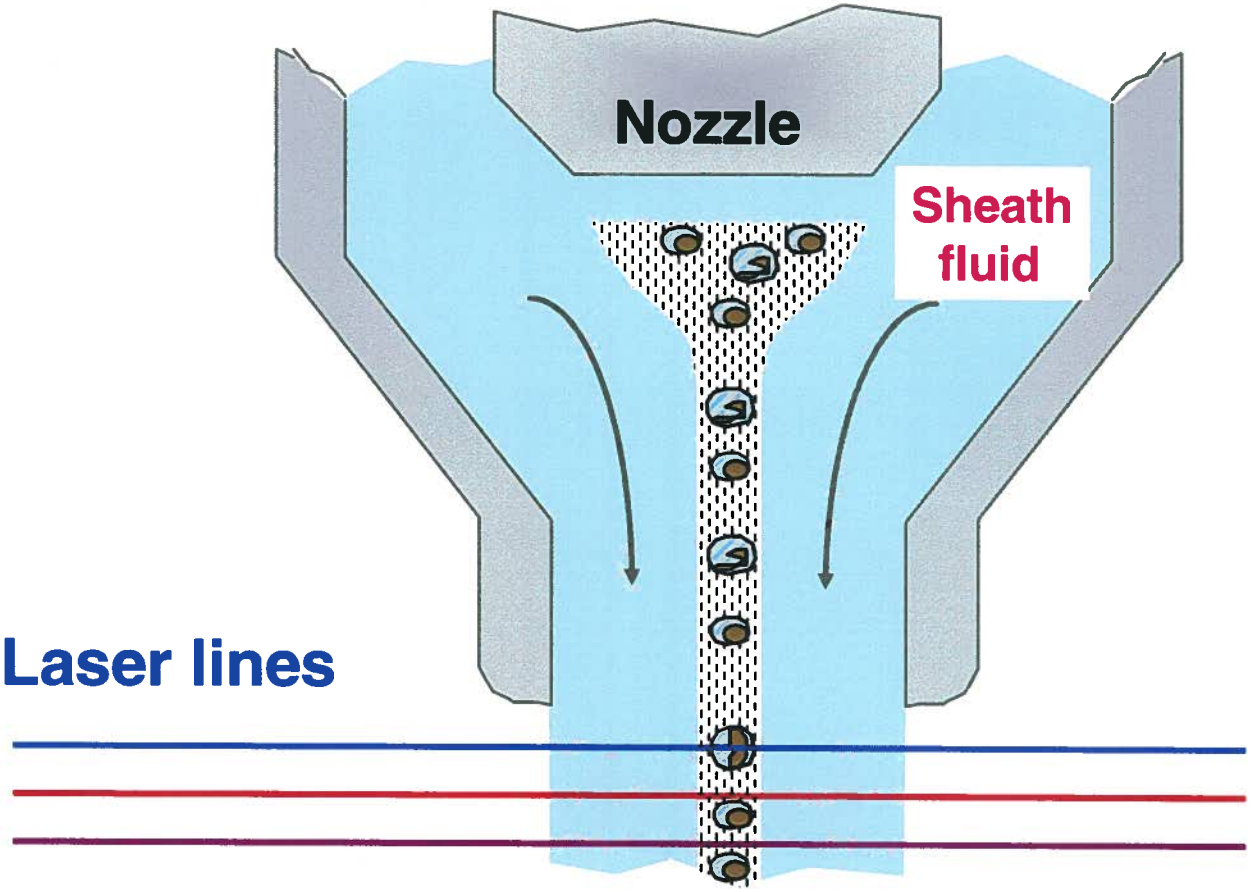
2 wavelengths – excitation and emission

The difference between the 2 wavelengths is the Stoke's Shift

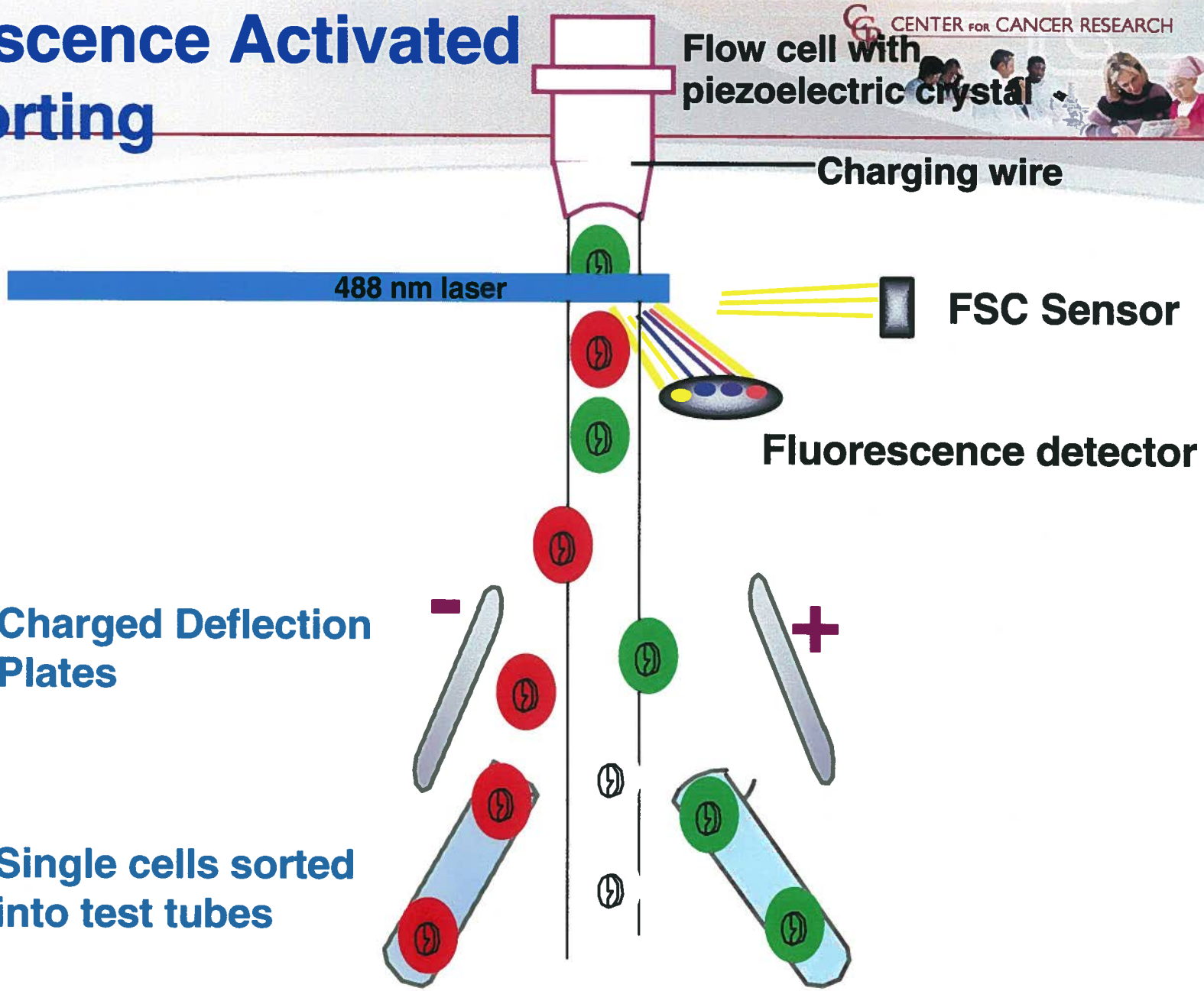
Fluorochromes for flow cytometry

Fluorochrome	Laser	Emission
FITC, Alexa 488	blue 488 nm	525 nm
PE	blue	575 nm
PerCP	blue	675 nm
PerCP-Cy5.5	blue	694 nm
PE-Alexa 700	blue	723 nm
PE-Cy7	blue	767 nm
APC	red 633 nm	660 nm
APC-Cy5.5	red	694 nm
APC-Alexa 700	red	723 nm
Alexa-Cy7	red	767 nm
Q dots	violet 405 nm	565,605,655,705 nm
CFP	purple 457 nm	485 nm
GFP	blue 488 nm	515 nm
YFP	blue	550 nm
dsRed	blue	610 nm
tomato, cherry	yellow 568 nm	610 nm

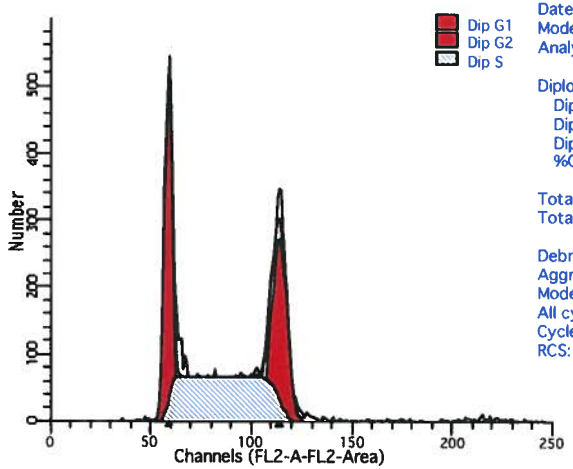
Flow Cell



Fluorescence Activated Cell Sorting



Cell cycle

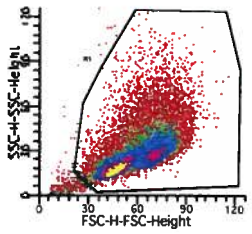


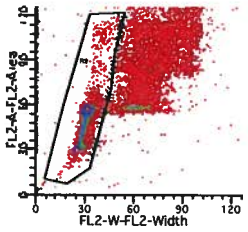
File analyzed: DNA.001
Date analyzed: 20-Sep-2001
Model: 1nn0n_DSF
Analysis type: Manual analysis

Diploid: 100.00 %
Dip G1: 27.69 % at 58.72
Dip G2: 29.62 % at 113.86
Dip S: 42.69 % G2/G1: 1.94
%CV: 3.11

Total S-Phase: 42.69 %
Total B.A.D.: 0.00 % no debris no aggs

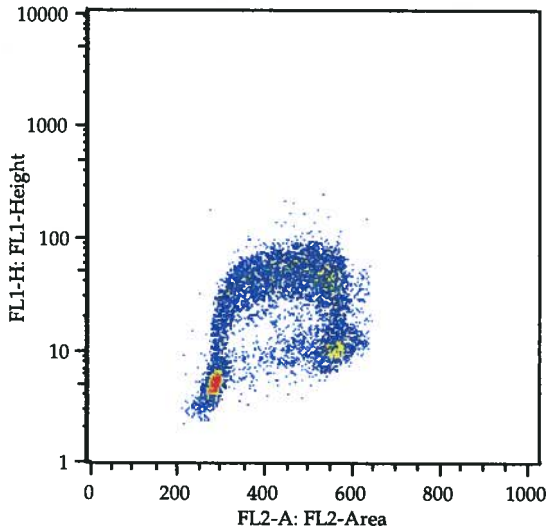
Debris: %
Aggregates: 0.00 %
Modeled events: 8434
All cycle events: 8434
Cycle events per channel: 150
RCS: 2.508

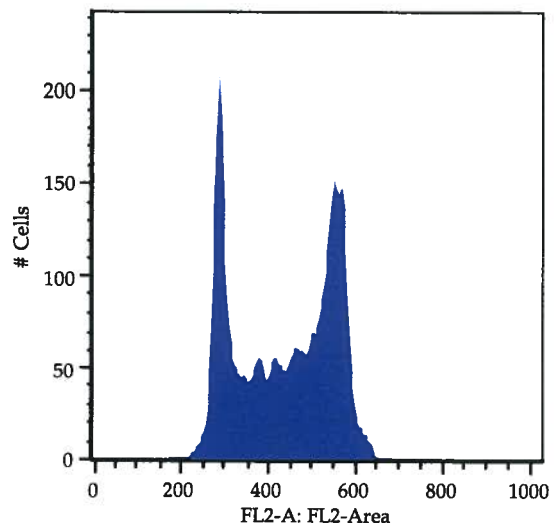




ModFitLT V3.0(PMac)

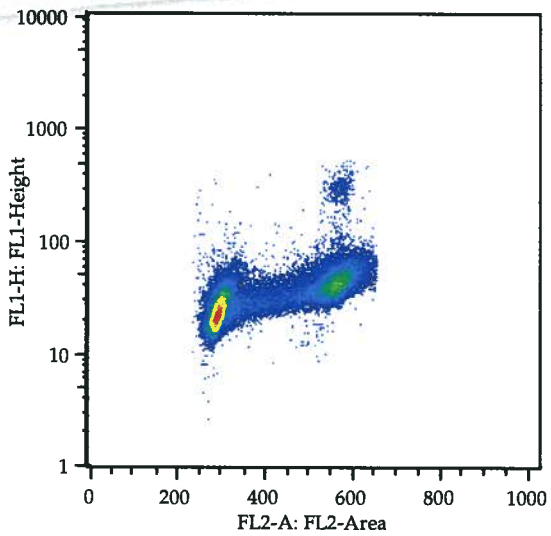
BrDU - S-phase labeling

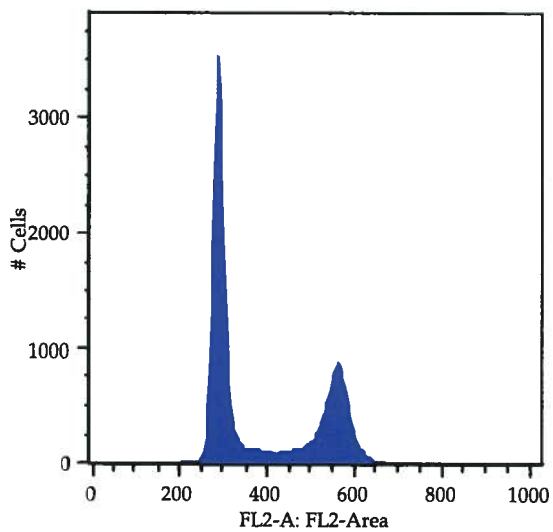




S-phase cells incorporating bromodeoxyuridine and then labeled with anti-BrDU

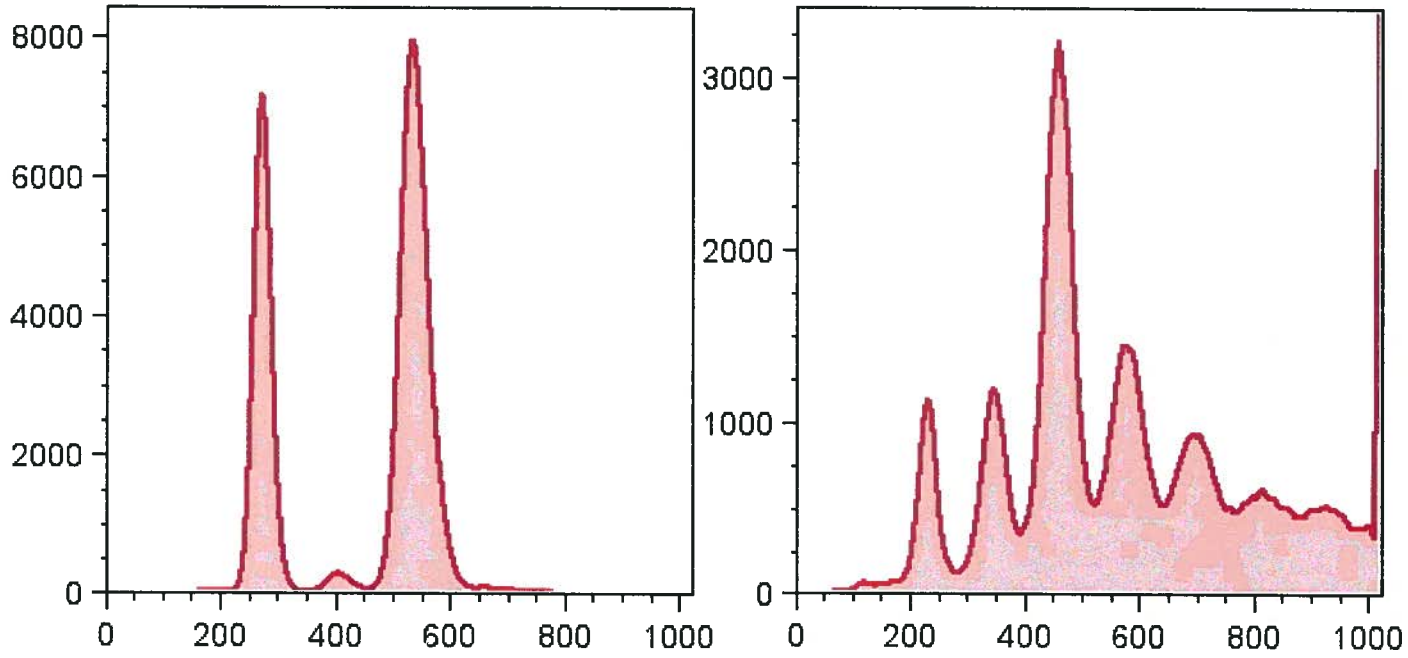
Mitotic cells





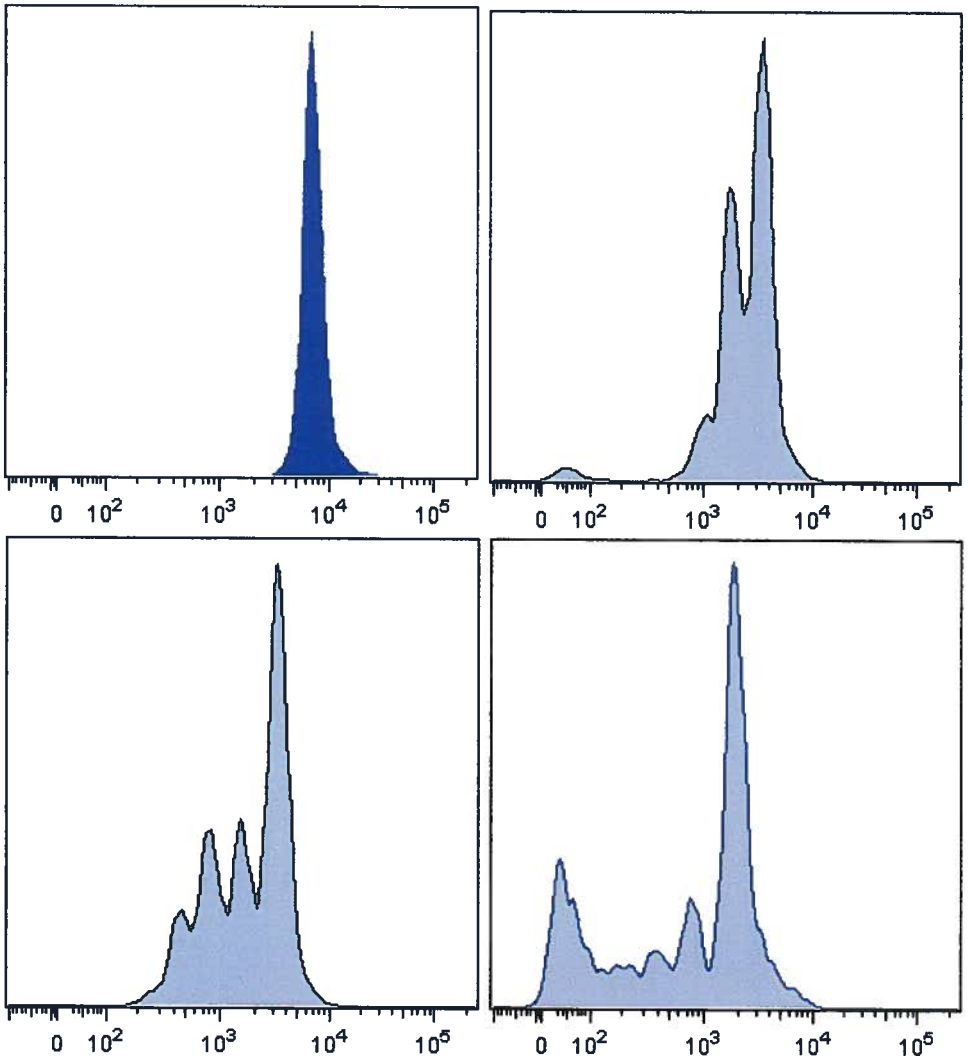
Mitotic cells labeled with anti-phosphohistone H3

Bacterial DNA



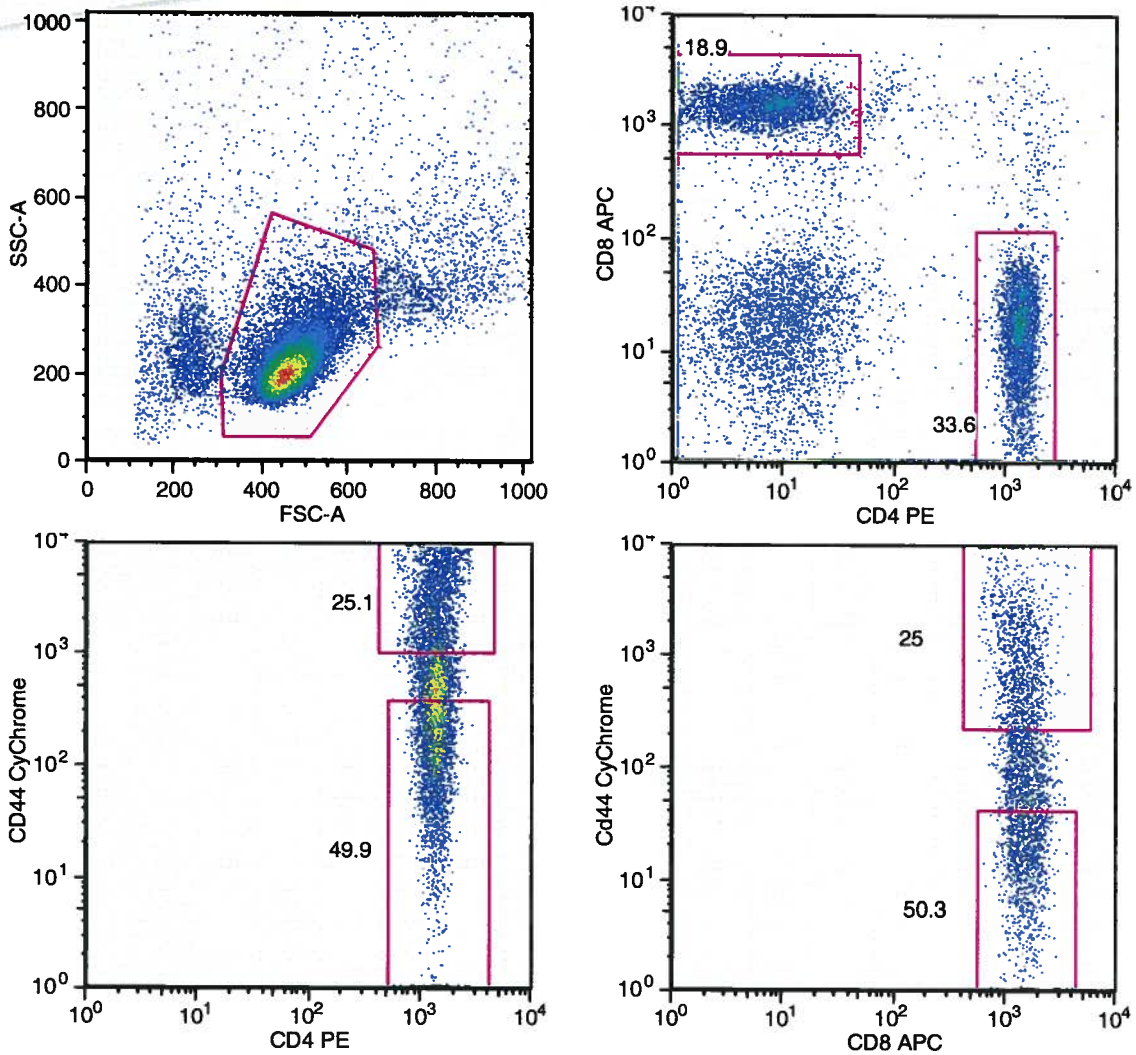
Synchronous and asynchronous replication in E coli using HO 33342

Cell tracking



Tracking cell proliferation with the membrane dye CFSE

Mouse spleen before sorting



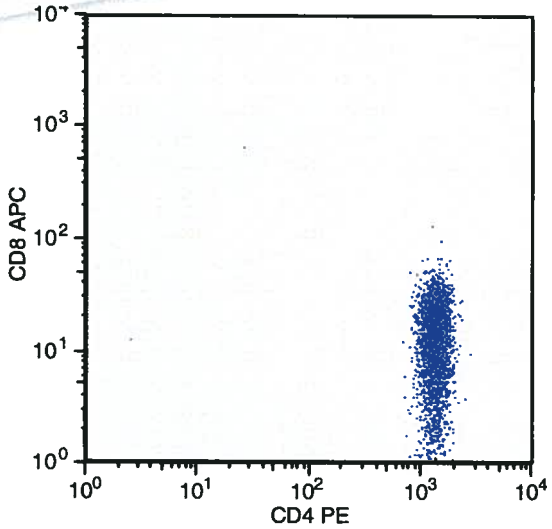
Mouse spleen

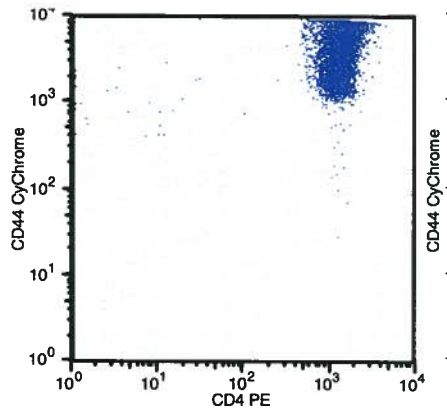
CD4 PE

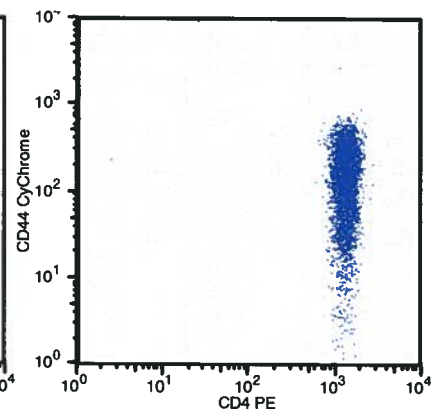
CD8 APC

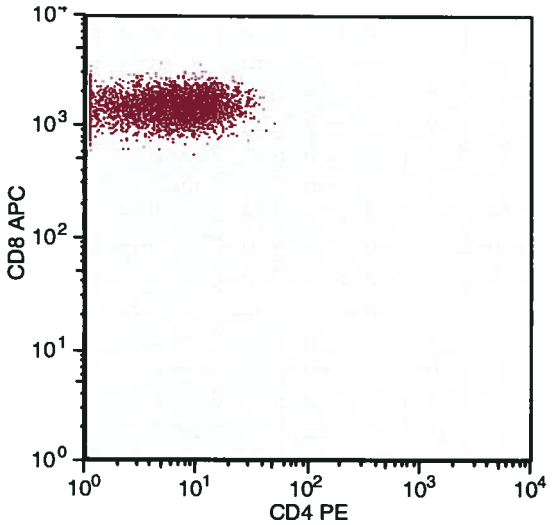
CD44 PE-Cy5

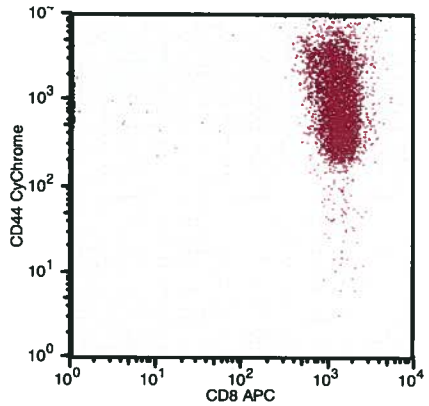
Mouse spleen - sorted

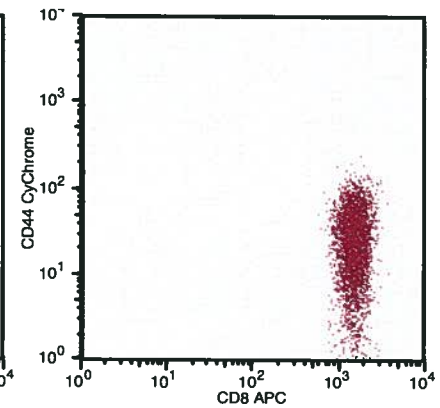




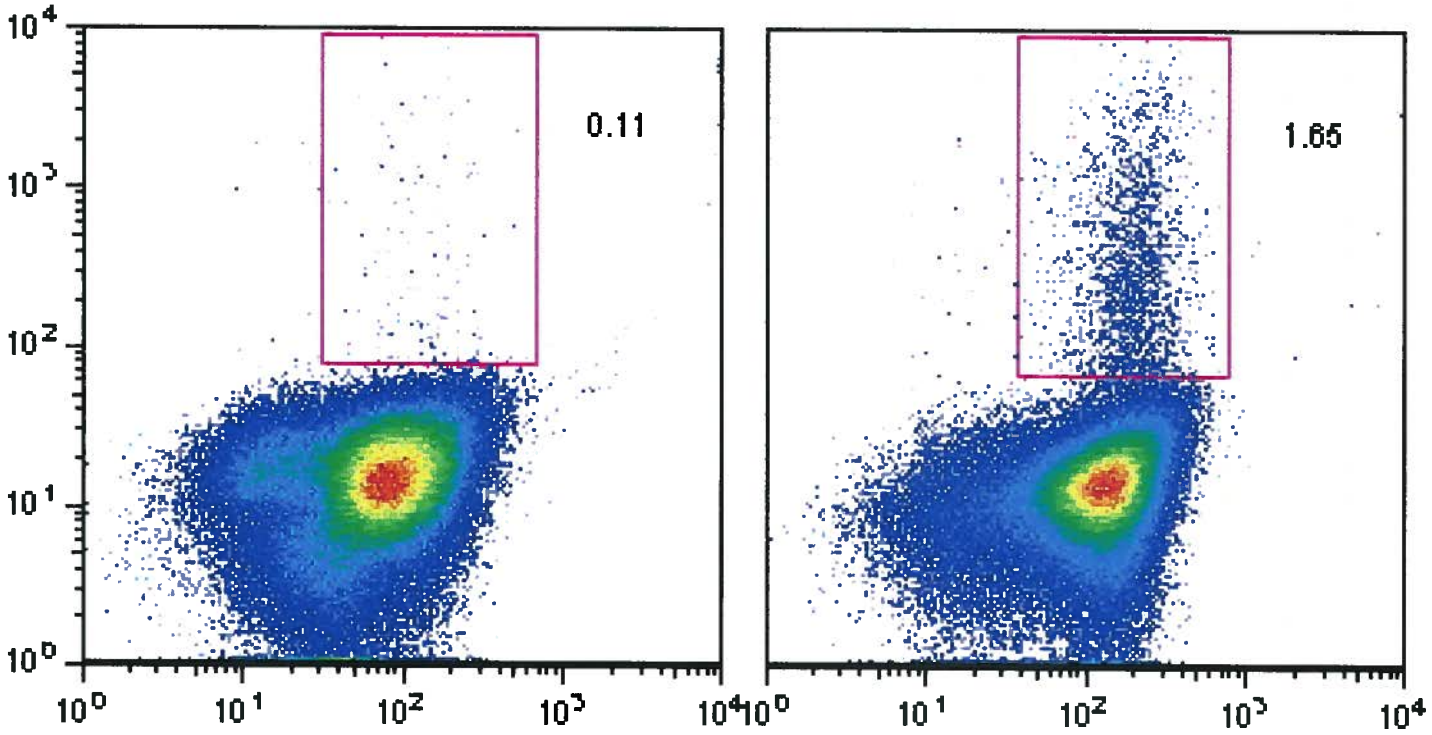








Intracellular cytokines



IL-17 expression in activated CD4 lymphocytes

Apoptosis

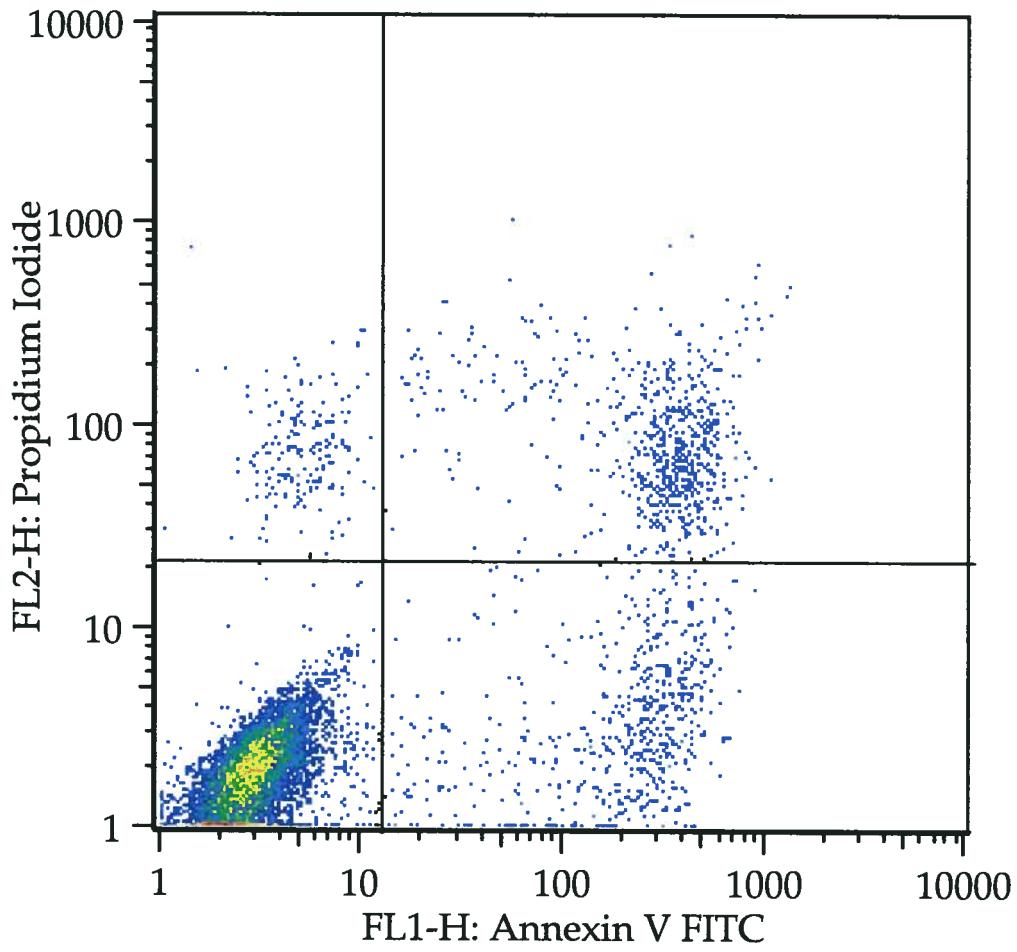
Annexin V- membrane phosphatidyl serine can be labeled with annexin V

Caspase 3- enzyme activity is measured by cleavage of a peptide substrate

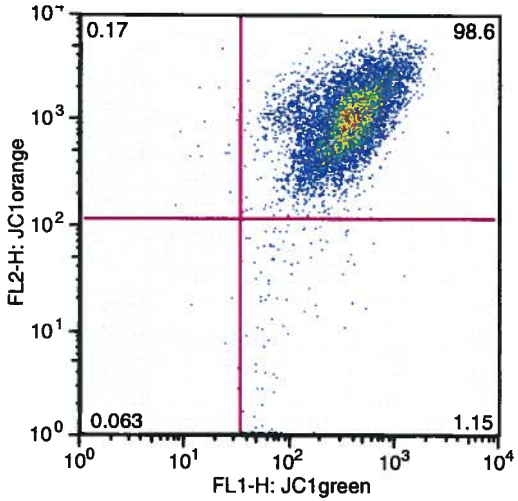
TUNEL-TdT-mediated dUTP-biotin nick end labeling – labels the broken ends of DNA strand breaks

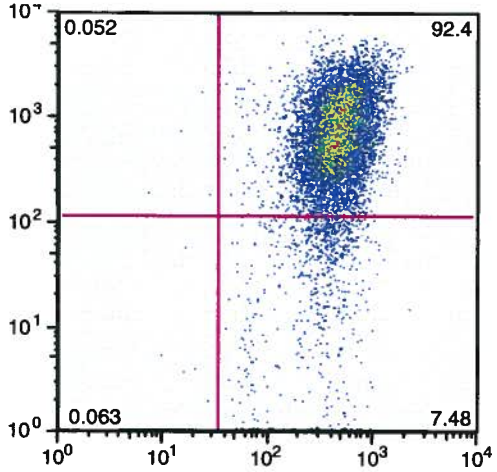
DNA degradation- low mw DNA fragments are extracted after fixation and an extra peak of apoptotic cells (sub G0/G1) can be seen after staining with PI

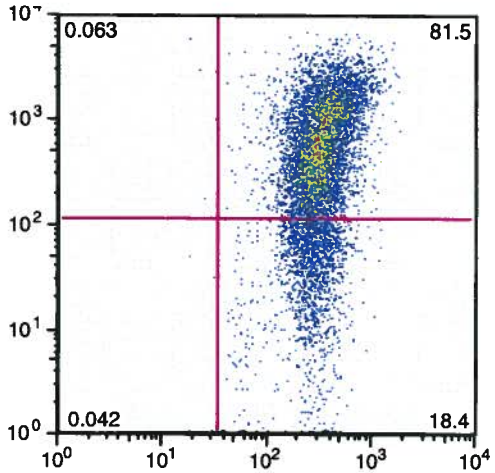
Annexin V

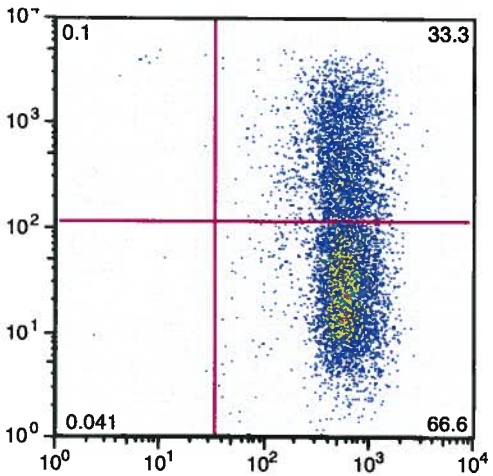

10000
1000
100
10
1
FL2-H: Propidium Iodide
1
10
100
1000
10000
FL1-H: Annexin V FITC

Mitochondrial membrane potential

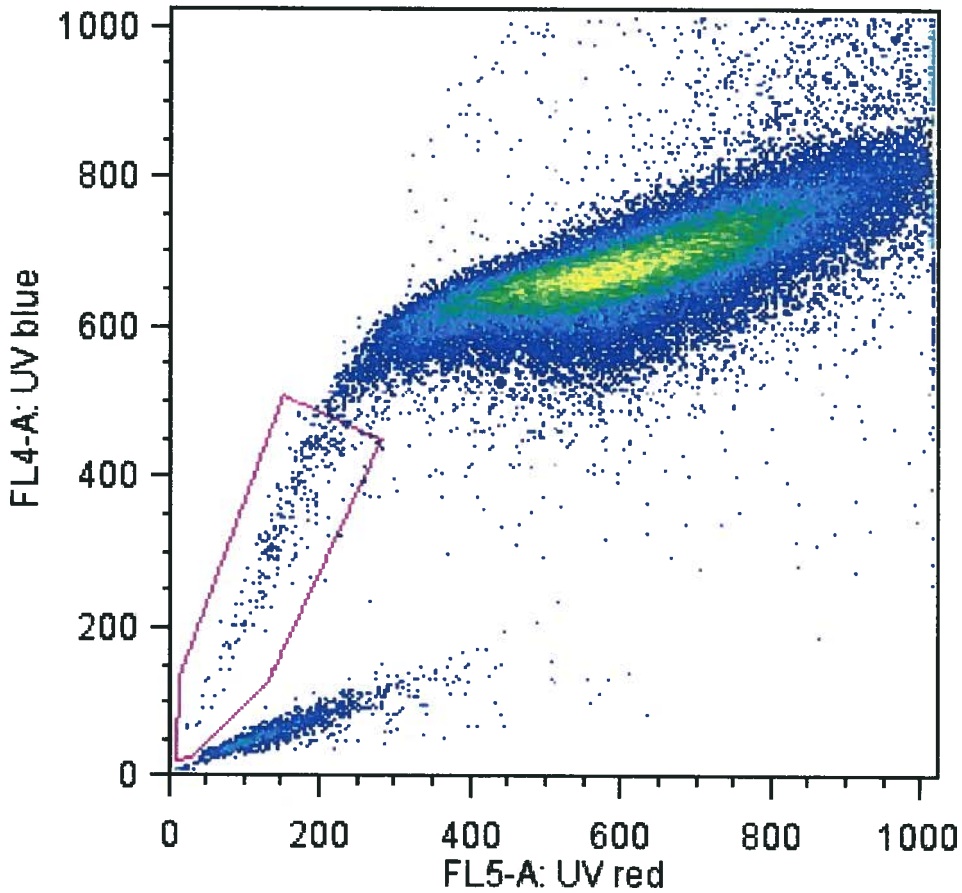








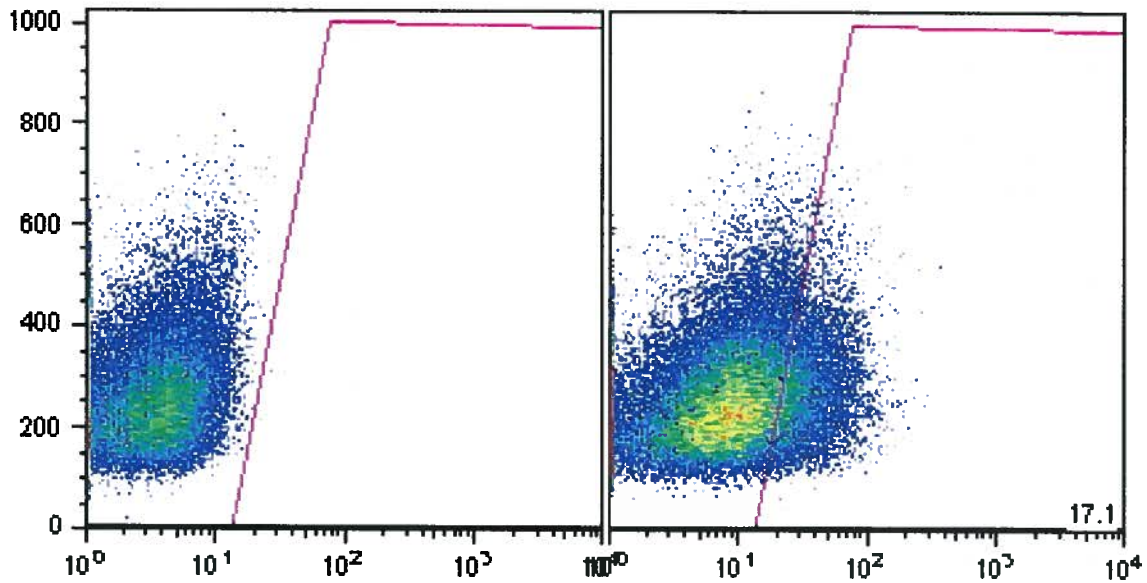
Stem cells - SP (side population)



Hoechst 33342 efflux

mediated by ABC transporter ABCG2

Stem cells



Unstained **CD133**

Resources

USEFUL FLOW CYTOMETRY RESOURCES (2009)

Fluorescence Spectrum Viewers

1. http://www.bdbiosciences.com/colors/fluorescence_spectrum_viewer//
   or go to: www.bdbiosciences.com,
   Support, Technical Resource Center, Flow Cytometry

2. http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html
   or go to: www.invitrogen.com,
   Applications, Cell and Tissue Analysis, Flow Cytometry

3. www.coulterflow.com

Choosing Fluorochromes

www.bdbiosciences.com/colors - download ŒSelecting Reagents for Multicolor Flow CytometryÕand fluorochrome reference chart

www.omegafilters.com - the Fluorescence Filters for Life Science Instrumentation catalog has an extensive list of fluorescent compounds with ex/em wavelengths

Resources

General Information

http://www.invitrogen.com/site/us/en/home/support/Tutorials.html
tutorials on fluorescence, flow cytometry

http://www.abdserotec.com/support/introduction_to_flow_cytometry-685.html
pdf handbook on flow cytometry

http://www.abdserotec.com/support/antibody_location_service-287.html
assistance with locating specific antibodies

www.coulterflow.com - Howard Shapiro's **Practical Flow Cytometry** in pdf format – more than you could ever want to know about flow

http://www.tissuedissociation.com/ - methods for dissociating solid tissues

Current Protocols in Cytometry, Current Protocols in Immunology – NIH library on-line

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Sue Sharrow, Bldg 10 EIB	sharrows@mail.nih.gov