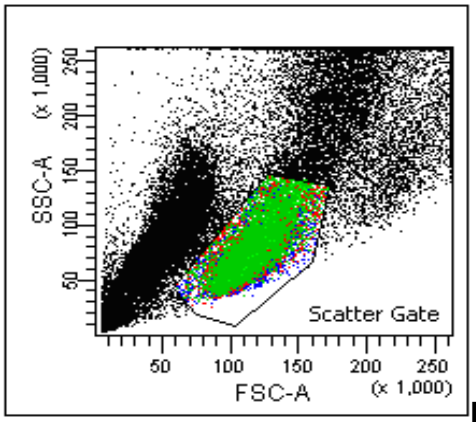
Uses of Flow Cytometry

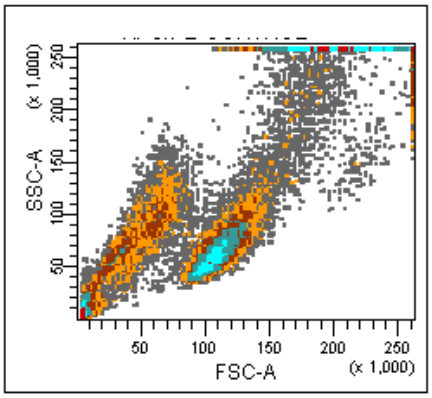
1. Multicolour analysis

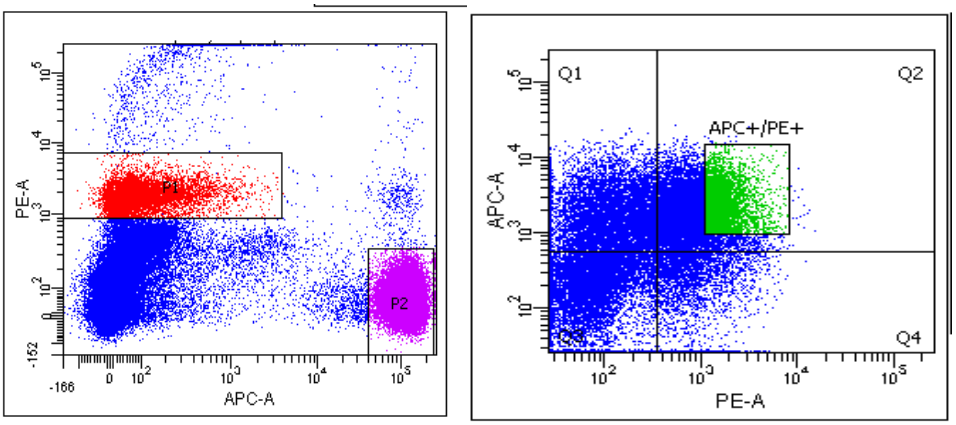
- **Fluorochromes**
- **Principles of surface antigen analysis**
- **Immunophenotyping**
- **Cytoplasmic antigen analysis**
  - simultaneously measure multiple parameters on a cell by cell basis
  - if more than two colors are being used, care must be taken to have relevant controls, dye combinations, electronic compensation and optical set-up

Dot Plot



Density Plot





2 colors parameters

2. Cell Cycle and Proliferation

- **Cell cycle analysis**
- **Analysis of Cellular DNA Content (PI and 7-AAD)**
- **Cell Proliferation Assays (BrdU)**

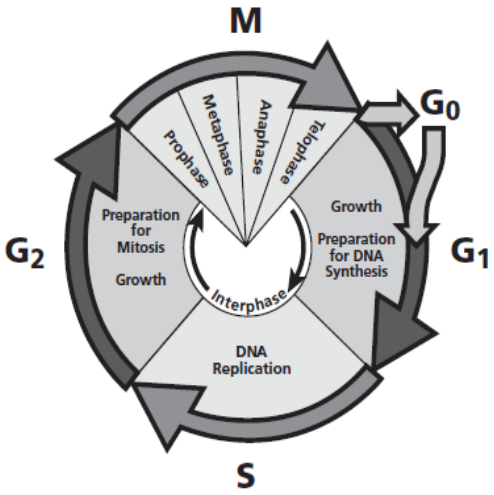


Figure 25. Cell Cycle phases

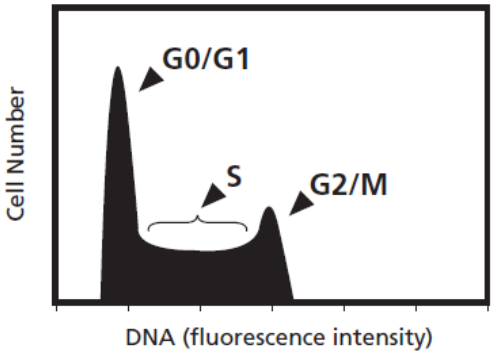


Figure 26. Analysis of cellular DNA content.

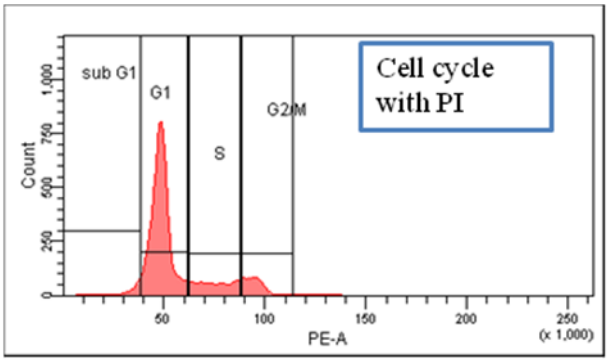
(source:BD Bioscience)

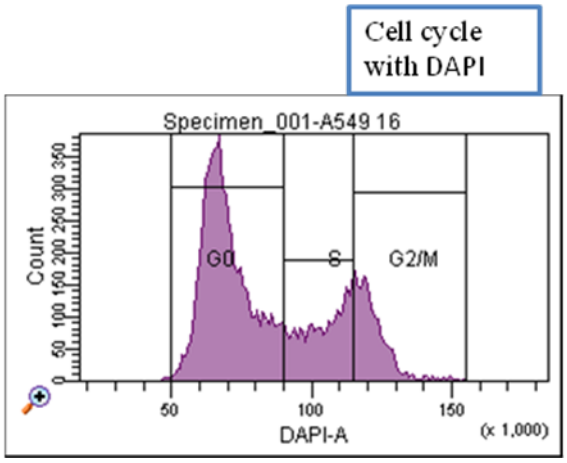
- Proliferation can measure cellular mechanisms involved in immunity, inflammation, hematopoiesia, neoplasia, and other biological responses.
- Cell growth, replication, and division in eukaryotic cells occur accordingly to a highly controlled series of events call the cell cycle

a. Analysis of Cellular DNA Content

- **Propidium Iodide**
  - For staining whole cells or isolated nuclei
  - PI intercalates into the DNA helix of fixed and permealized cells
  - For staining of DNA only, use RNase to remove RNA
  - For staining of cells in late stages of apoptosis
  - Discriminator of live and dead cells

- **7- aminoactinomycin D (7-AAD)**
  - DNA-specific dye used for staining fixed and permeabilized cells
  - Determine DNA content profiles of cell populations
  - Use together with BrdU staining
  - Readily stain dead cells
  - Discriminator of live and dead cells

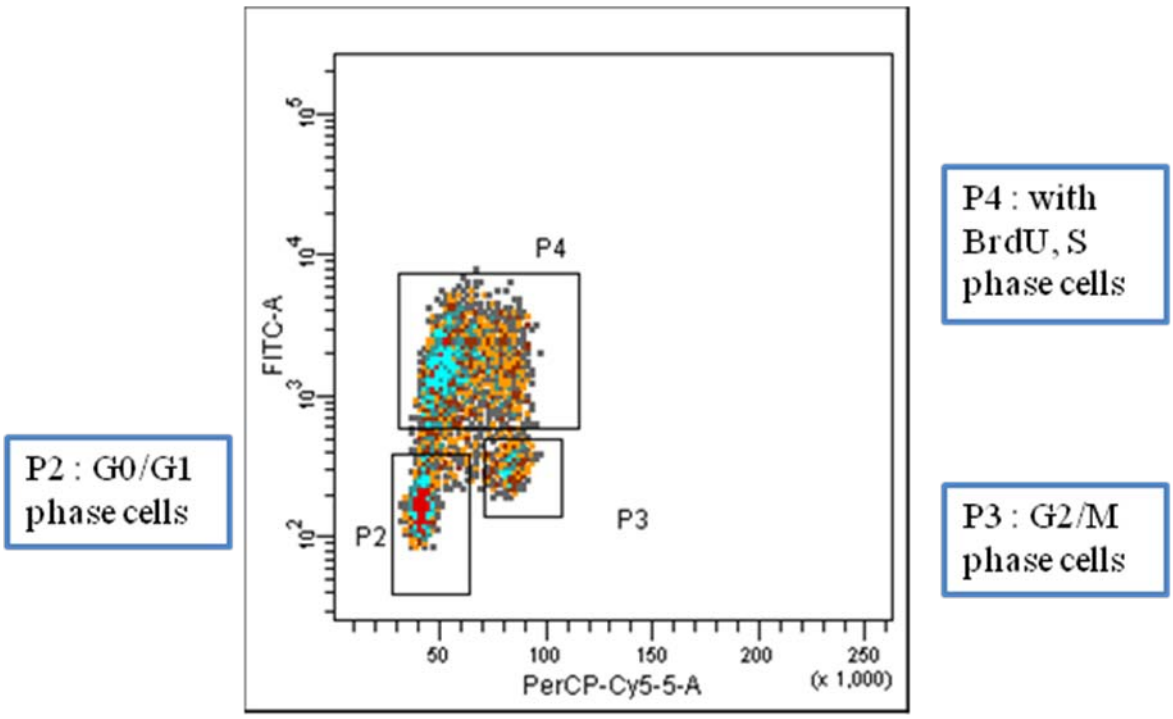




b. Cell Proliferation Assays

Determination of S Phase Activity using Bromodeoxy- Uridine

- **BrdU**
  - Analog of the DNA precursor thymidine during S (DNA synthesis phase
  - Readily detected by anti-BrdU-specific antibodies (that do not recognize thymidine)



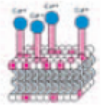
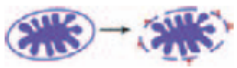
3. Immunology

- **Rare event analysis**
- **Dendritic cells**
- **Stem cell/progenitor cells**

  - The coupling of monoclonal antibody technology to characterize cells based on cell surface expression
  - Many of the antibodies identify cells with important functions or cells of distinct lineages, immune cells can be analyzed for distribution
  - Lymphocytes can be sorted into subsets based on cell surface characteristics and then tested for immune function
  - More recent flow cytometric assays, include assays for intracellular cytokine production after stimulation in vitro and direct measurement of antigen specific cells using tetramers)

4. Apoptosis

- **Apoptosis or Programmed cell death**
- **Normal physiological process that occurs during embryonic development and in the maintenance of tissue homeostasis**
- **Detection of drug treatment to induce death etc**

Feature Measured	Assays	Key Features
Phosphatidylserine Exposure 	**Annexin V binding assay** • Single conjugates • Annexin V Kits	• Detects early Apoptosis markers • Quick and easy • Flow cytometry or Immunofluorecsence application
Mitochondrial Changes 	• BD Mitoscreen Kit	• Fast, easy, single cell resolution by flow cytometry or fluorescent microscopy
Caspase Activation 	• Caspase Activity Assay Kits and Reagents	• Quick and easy, uses spectrofluorometry
	• Active Caspase -3 immunoassays (BD CBA, ELISA)	• Specific, quantitative, flow cytometry and ELISA application
DNA - Fragmentation 	• APO-BrdU™ TUNEL ASSAY • APO-DIRECT™ TUNEL ASSAY	• Works adherent cells, single cell resolution in conjuunction with cell cycle analysis by flow cytometry

(source:BD Bioscience)

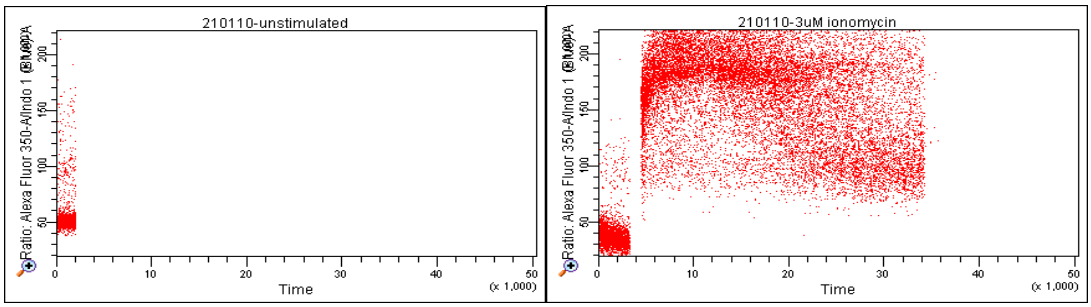
5. Functional Assay

- Calcium Flux measures intracellular Calcium Concentration

  - Flow cytometry can be used to measure the concentration of intracellular free calcium ions which provides information about the number of responding cells as well as the relative magnitude of the response to a given stimulus.

  - In their resting state, eukaryotic cells maintain an internal Ca^{++} concentration far less than that of the extracellular environment. Elevation in intracellular Ca^{++} concentration is often used as an indicator of cellular activation in response to a stimulus. Calcium flux is also an indicator of whether the cells in a population remain functional after exposure to a drug or other compound.

  - Several fluorescent dyes measure intracellular Ca^{++} levels. For most of them, the amount of Ca^{++} entering a cell is indicated by a change in fluorescence emission. For example, the emission spectrum of indo-1 changes from blue to violet upon binding to Ca^{++}. The ratio of violet to blue fluorescence is independent of the amount of dye within the cell.



unstimulated **stimulated (positive control)**

6. Haematology

- Used extensively in Haematology for the listing of blood cell sub-populations
- Sorting allows purification of cells populations in blood samples

7. Genetics

- Sorting for the production of chromosome-specific DNA libraries
- paints for cell cycle analysis and ploidy determinations.

8. Oncology

- In analysis of hematological malignancies
- Surface immunophenotyping, an established diagnostic procedure in clinical laboratories, continues to develop with the introduction of newer surface markers, instrumentation and analysis platforms. Increasingly this is being complemented by newer methods that probe underlying molecular mechanisms that influence treatment sensitivity or biological aggression, or track the existence of minimal residual disease.
- Potential to monitor residual disease in solid tumour patients through the detection of rare circulating tumour cells in the peripheral blood or bone marrow.

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