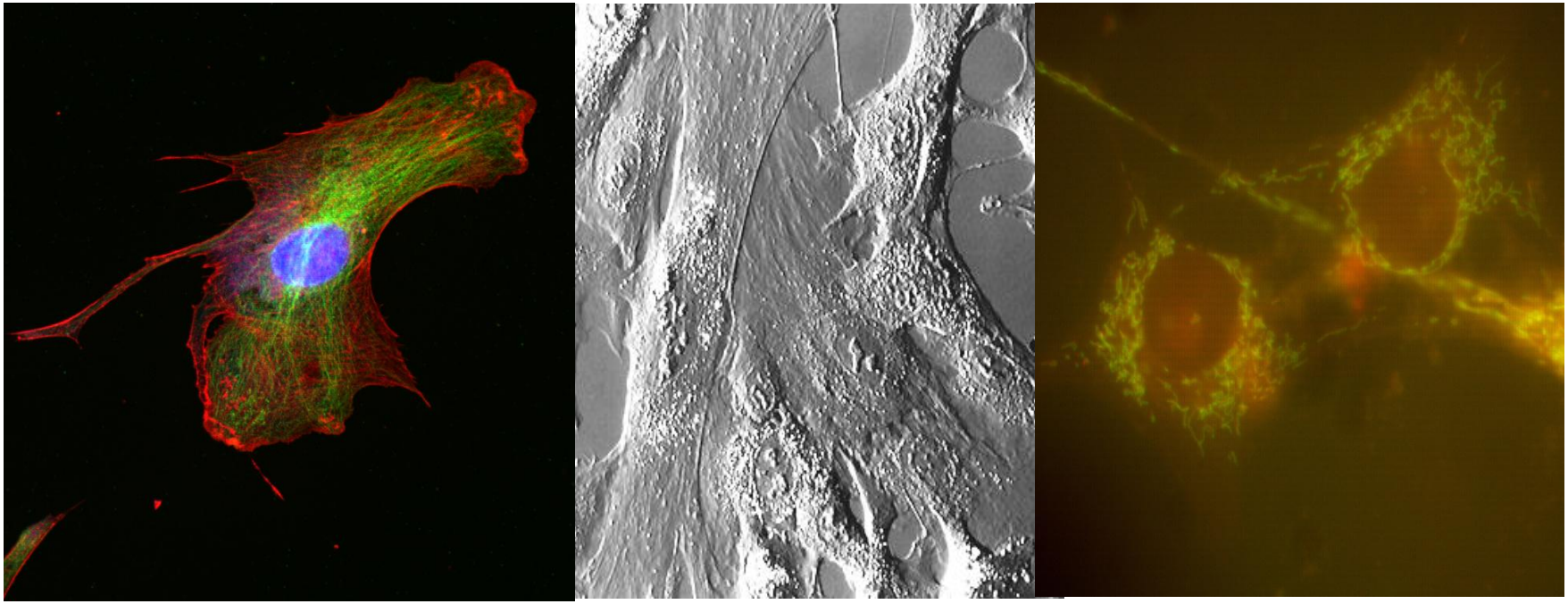
Bioc 315

Cell Culture



The culture of cells (animal, plant, insect) *in vitro.*

Requirements for cell culture

Minimum Requirements	Useful Additions
-Sterile area, clean and no through traffic -Separate from animal house and micro labs -Incubator -Biological Safety Cabinet (BSL1 or BSL2) -Microscope - Cell Store (liquid nitrogen freezer) -Water bath -Centrifuge -Fridge/freezer -Storage areas for: Liquids and chemicals (ambient; +4°C;-20°C) Glassware and Plastics Small items – pipettes, haemocytometers etc… Specialised equipment Sink Prep and wash up area	-HEPA filtered air-con -Temperature control (24 hr) -Electrical supply back-up (standby generator) -Sterilizing area (or room) -Cylinder storage area -Autoclave -Controlled cell freezer

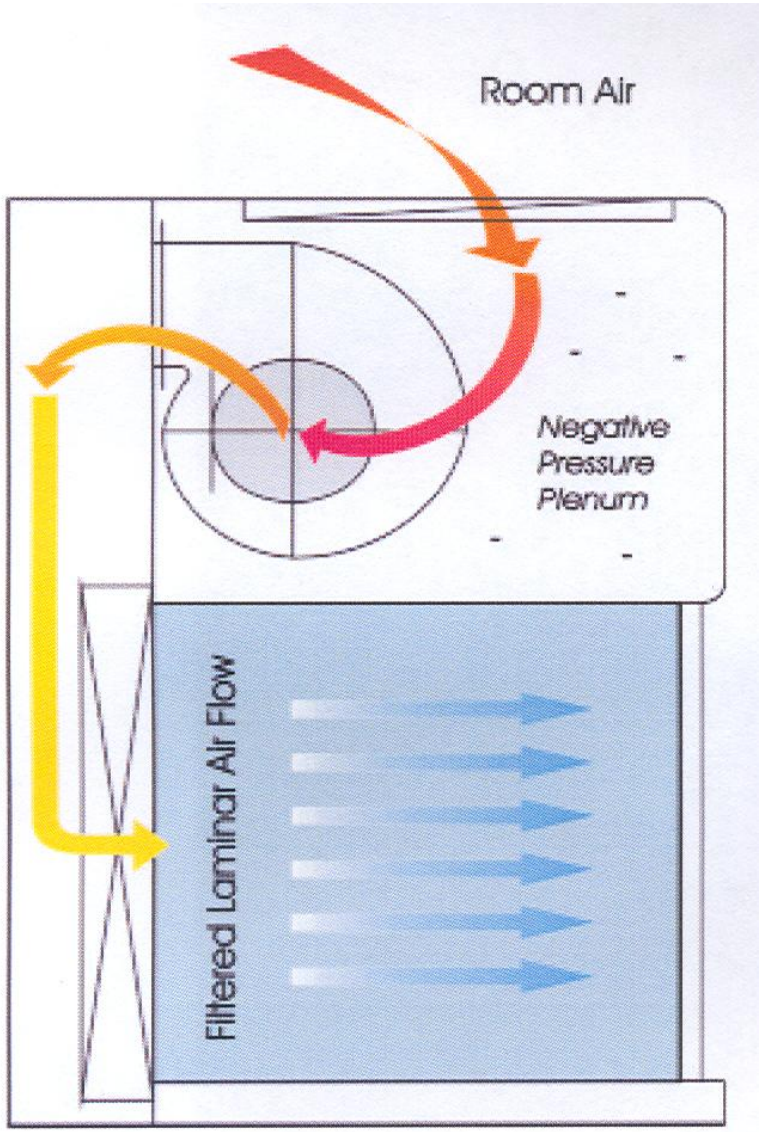
Sterile work areas – Laminar Flow Hoods

BSL-1

BSL-2

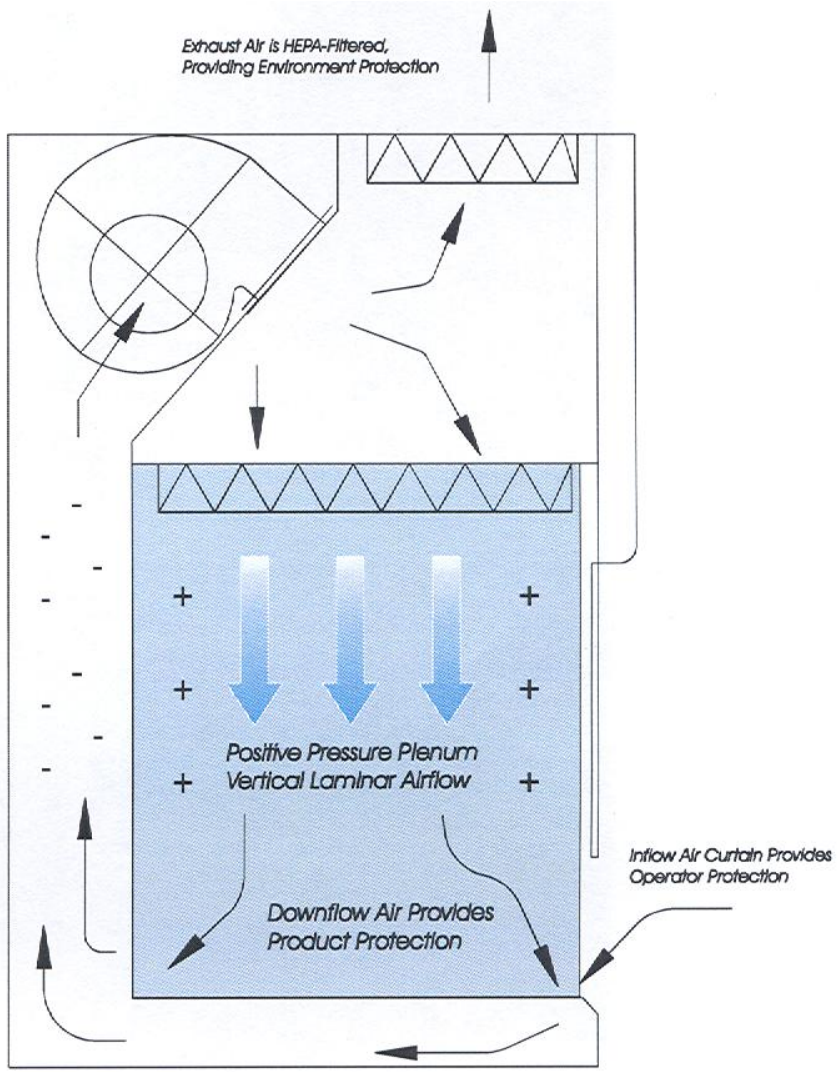
Sterile work areas – Biological Safety Cabinets

BSL-1



Typical Horizontal Laminar Flow Cabinet Airflow

BSL-2



Airflow Profile of a Typical Class II Biohazard Safety Cabinet

Growing cells…

To grow cells in culture you need to provide:

- correct nutrients
- correct pH
- temperature control
- gas control
- humidity control
- sterile conditions

Correct nutrients.

Different cells need to be provided with distinct mixes of nutrients to keep them alive (viable) in culture.

To achieve this we use two main ingredients:

- media (salts, buffers, vitamins, amino acids)
- serum (hormones, attachment factors, buffer)

Growing cells…

What else?

Well, we need to keep our cells free of infection. To achieve this we use antibiotics in the media.

Penicillin / Streptomycin (pen/strep) are the most commonly used.

Antifungal agents can also be used (eg Fungizone).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Growing cells…

We now know the nutrients we use to grow cells in, but how do we make this up…

…we typically use:

90% media
10% FCS
1% pen/strep

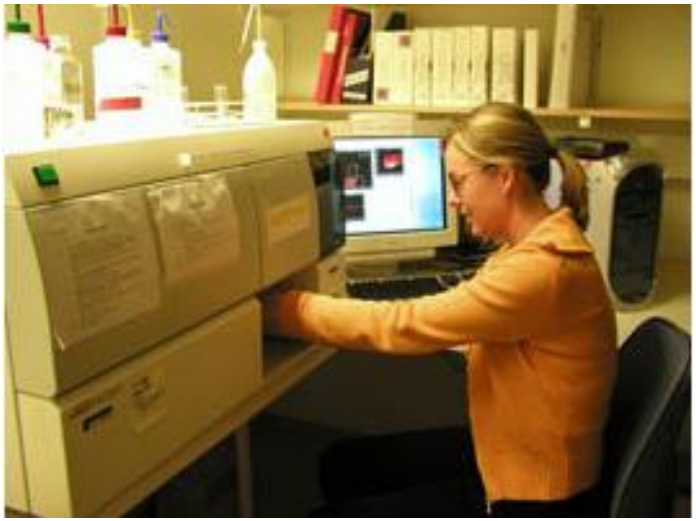
NB. This is more than 100%, but as long as you are consistent with the way you work, this is OK.

Growing cells…

Cells also require a controlled environment in which to grow. To achieve this we use an incubator, in which we can control temperature, humidity and the % CO_2 in the atmosphere.

Flow cytometry

- a technique for counting, examining, and sorting microscopic particles suspended in a stream of fluid.



•allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of single cells flowing through detection apparatus.

FACS: Fluorescence Activated Cell Sorting

Flow cytometry

1: The tissue sample is broken up into single cells and held in a test tube. The cells are drawn up from the test tube and pumped into the flow chamber.

2: Cells flow through the flow chamber one at a time at about 500 cells per second.

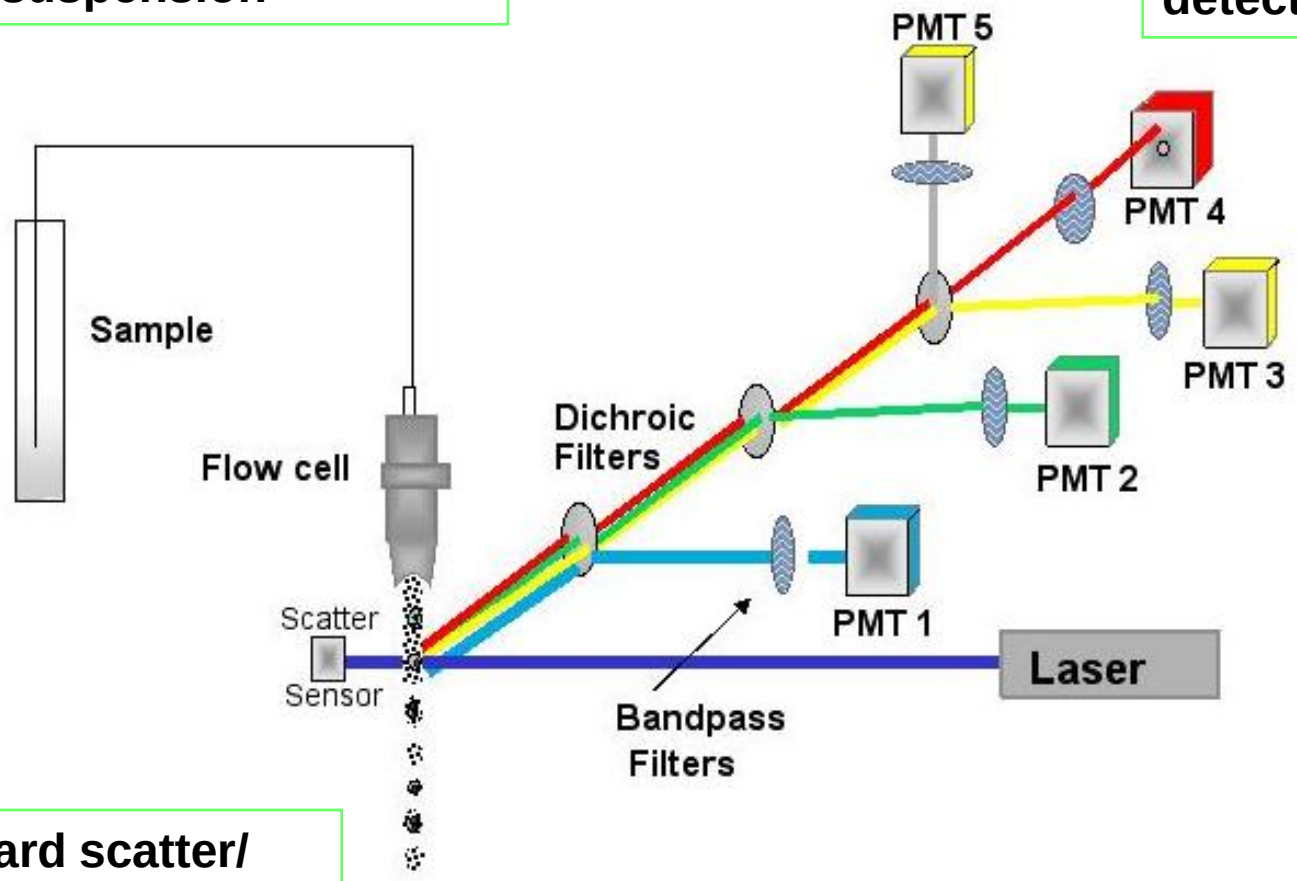
3: A small laser beam hits the cells as they pass through the flow chamber. The way the light bounces off each cell gives information about the cell's physical characteristics: Forward scatter tells you size of the cell; Side scatter tells you granularity.

4: Filters direct the light emitted by fluorochromes. As the cells pass through the laser, fluorochromes on the cells absorb light and then emit a specific color of light. Detectors collect different colors of light emitted.

5: Electronic processing: Data from detectors is sent to a computer and plotted as a dot plot or histogram.

Sample must be single cell suspension

Light emitted due to presence of fluorochromes is picked up by colour detectors

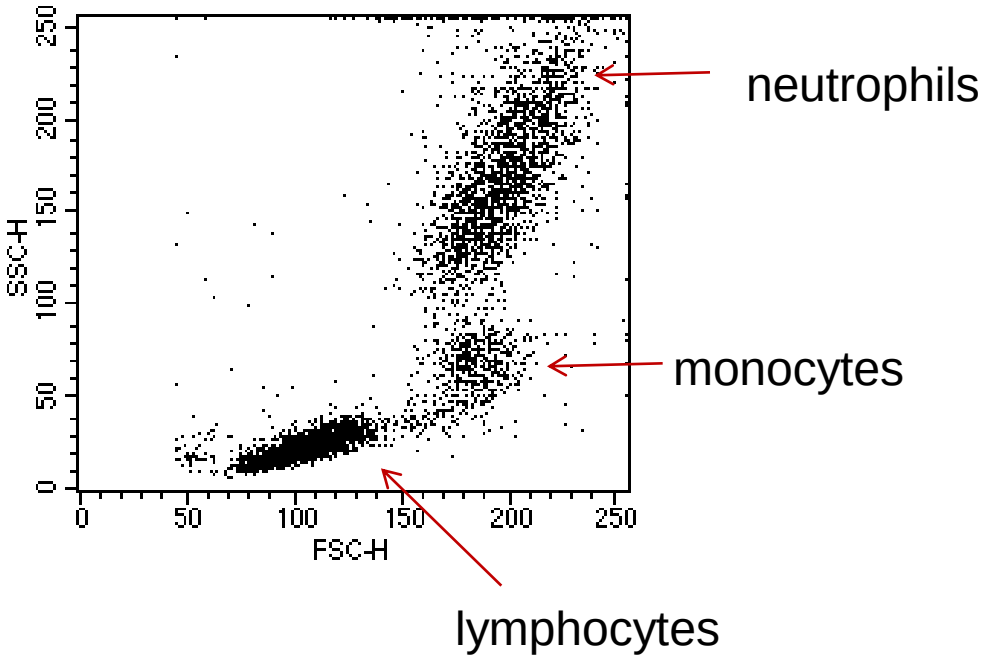


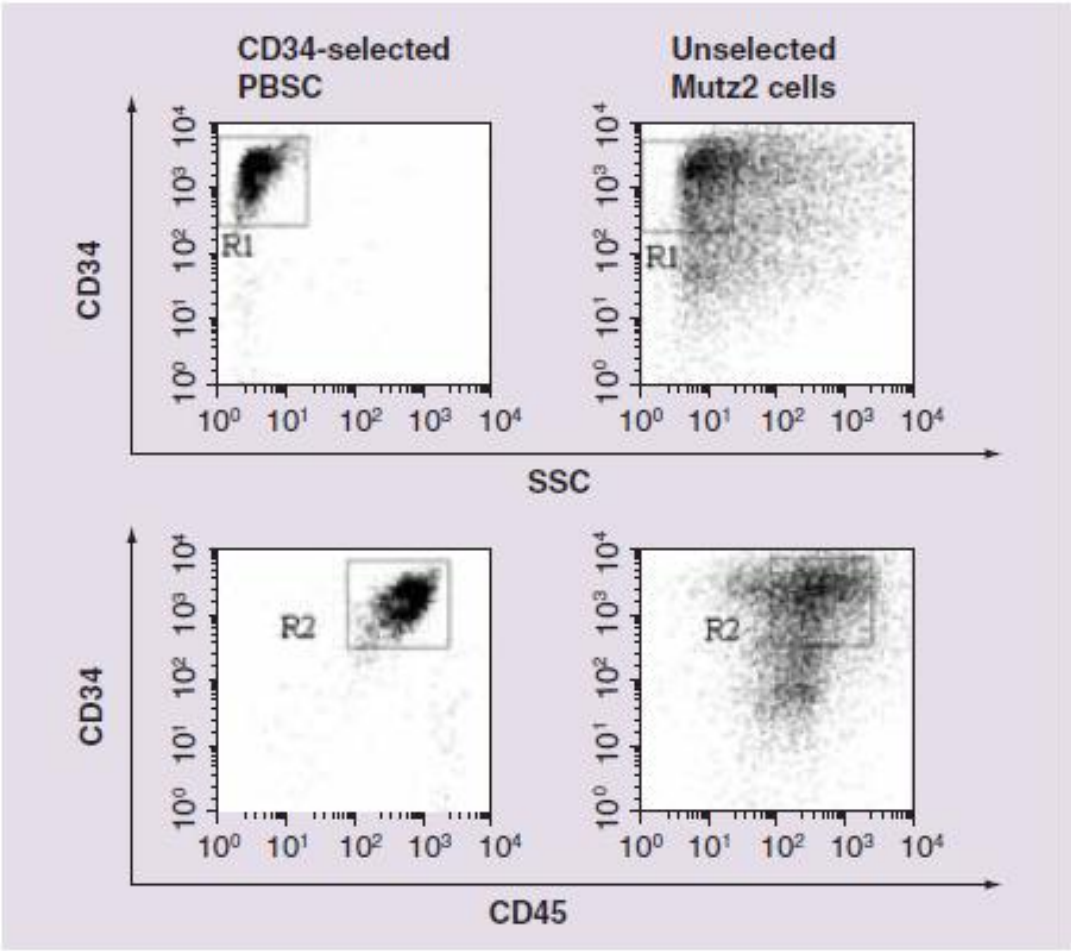
Forward scatter/ Side scatter is picked up by light detector (sensor)

High throughput: Flow cytometry allows analysis of multiple parameters at the same time.

Blood cell types: FSC vs SSC

lysed whole blood





The main population of both CD34+ HPC and unselected Mutz2 cells expressed the cell-surface antigens CD34 and CD45, and is characterized by low granularity. PBSC: Peripheral blood stem cells; SSC: Side scatter.

From Wiehe, Niesler 2006, Regenerative Medicine

Ploidy analysis (DNA content)