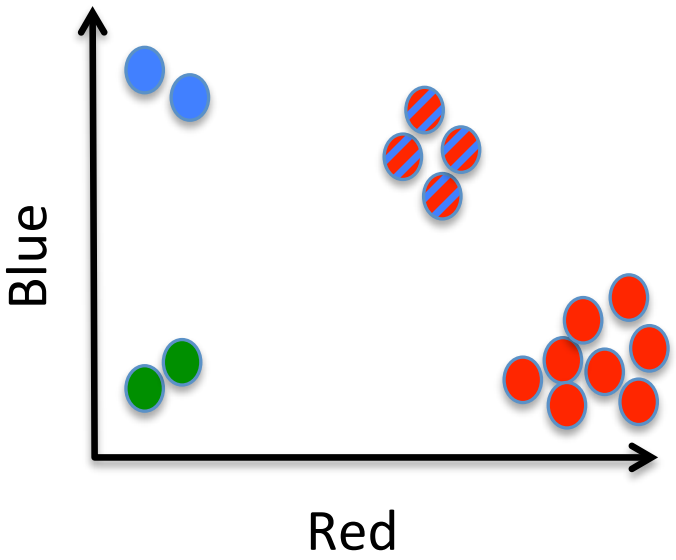
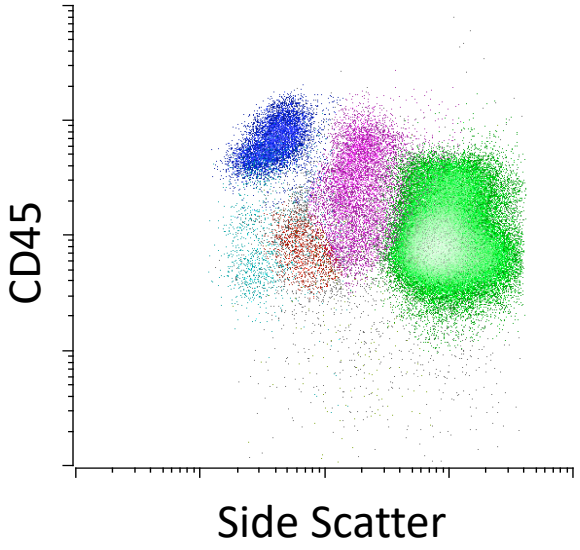
What is flow cytometry?





- Flow cyotmetry is a technology used to characterize different cellular populations in a mixture of cells

- It is a tool often used in the diagnosis and classification of hematopoietic neoplasms.

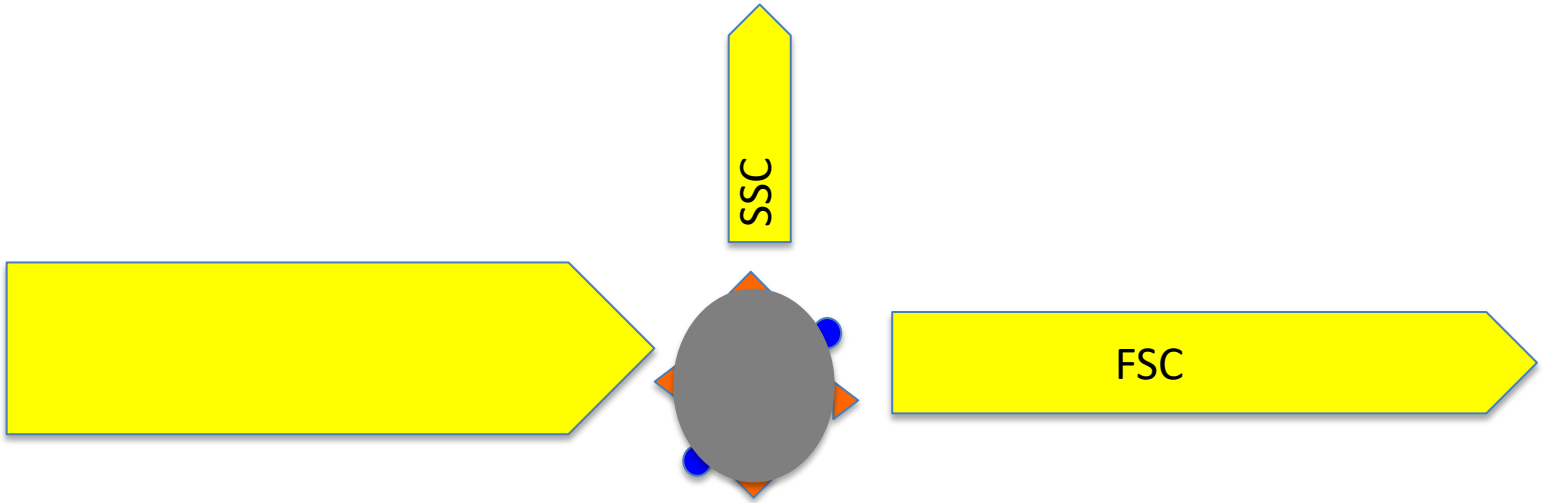
- Flow cytometry uses the ability of cells to scatter light to measure physical properties of a cell and can also be used to detect antigens expressed on or in a cell by staining a mixture of cells with fluorescently labeled antibodies

What sample types are appropriate for flow cytometry?

- Clinical flow cytometry requires fresh specimens
  - Ideally <24 hours
  - May submit tissue in nutrient media (RPMI)
  - Do not submit in a fixative (formalin)
- Flow cytometry requires that cells are in a liquid suspension
  - Liquid samples
    - Blood
    - Bone marrow aspirates
    - Body fluids: CSF, ascites fluid, etc
  - Solid samples can be used for flow cytometry if they are disaggregated (chopped) and suspended in fluid
    - Lymph node
    - Tissue biopsy

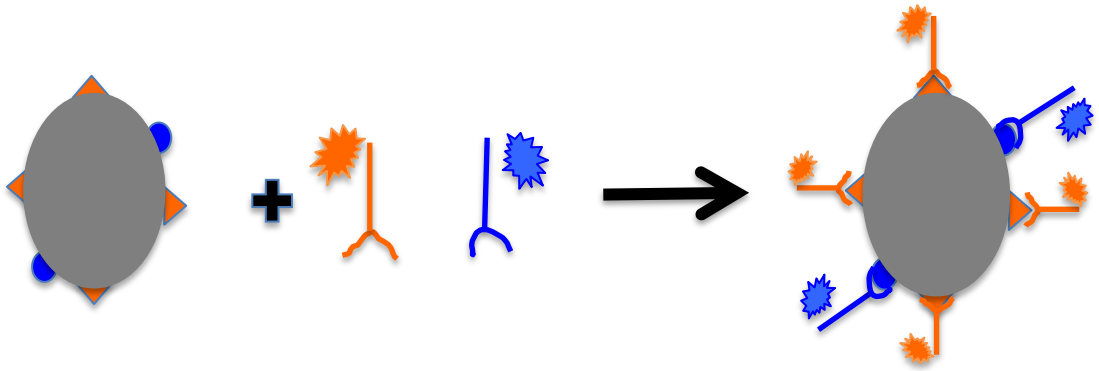
How does flow cytometry work?

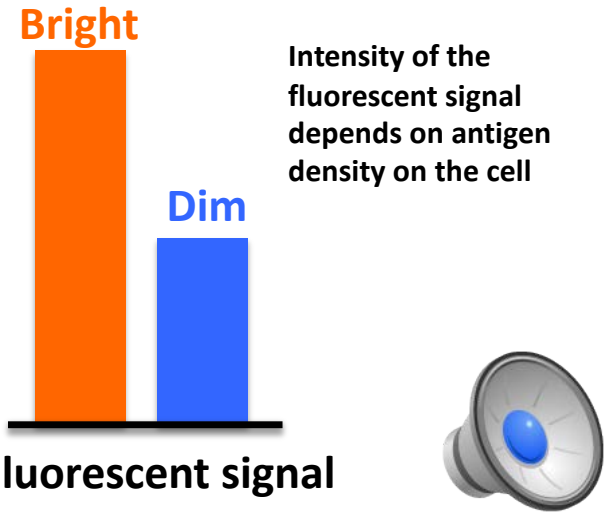
- Flow cytometry harnesses the ability to scatter light to measure **physical properties** of a cell
  - Size: Forward scatter (FSC)
  - Cellular complexity or granularity: Side scatter (SSC)



How does flow cytometry work?

- Cells express characteristic markers (**antigens**)
- Pattern of antigen expression = **immunophenotype**
- Antigens can be detected by **antibodies**
- Flow cytometry can be used to determine the immunophenotype of a cell by staining that cell with antibodies associated with fluorescently labeled tags





Immunophenotyping in hematopoietic neoplasms

- Antigen expression is well characterized for hematopoietic populations
  - Normal maturation
  - States of disease
- Immunophenotyping is an important part of characterizing hematopoietic neoplasms

Markers of immaturity
CD34, TdT

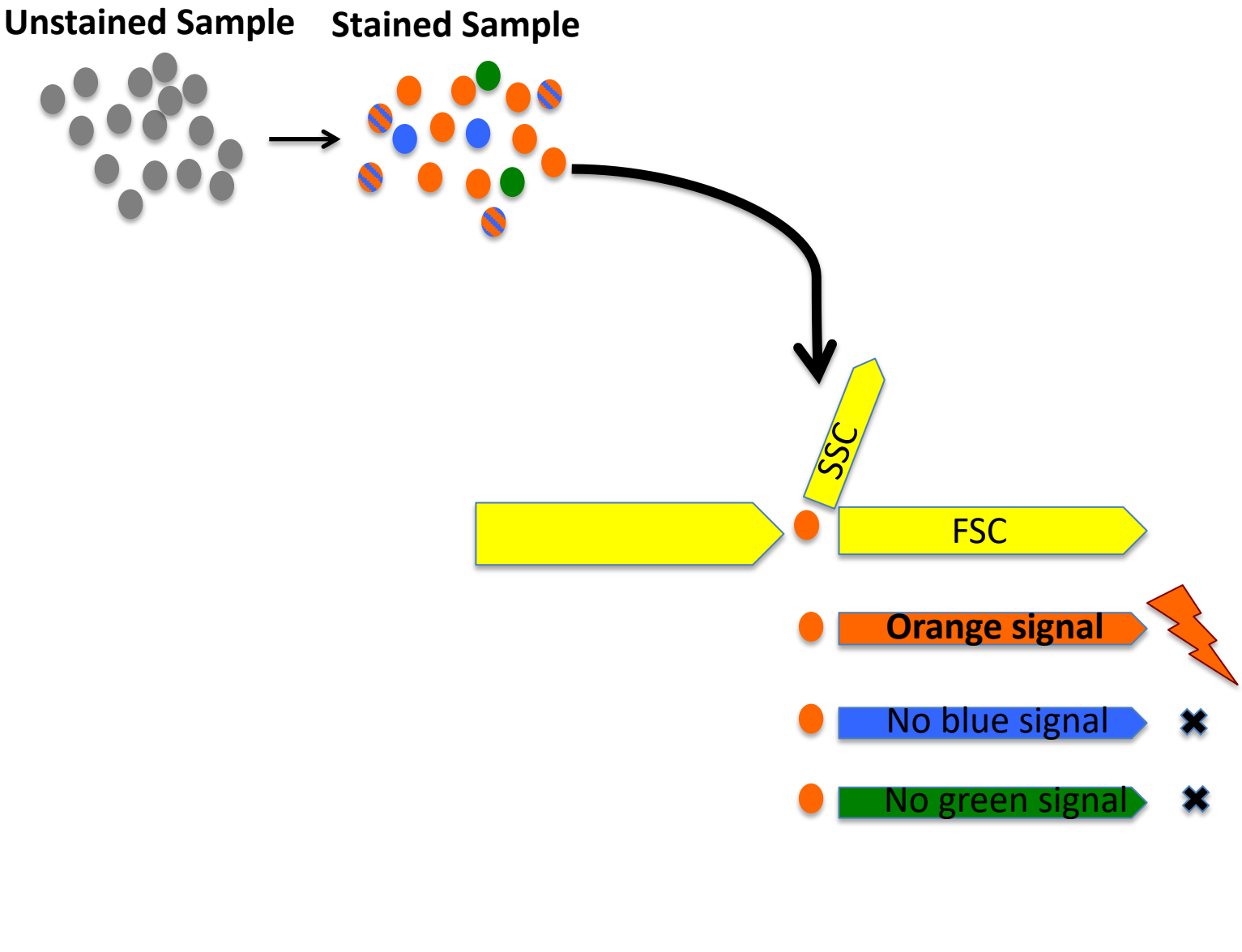
Myeloid markers
CD117, CD13, CD33, CD15, myeloperoxidase

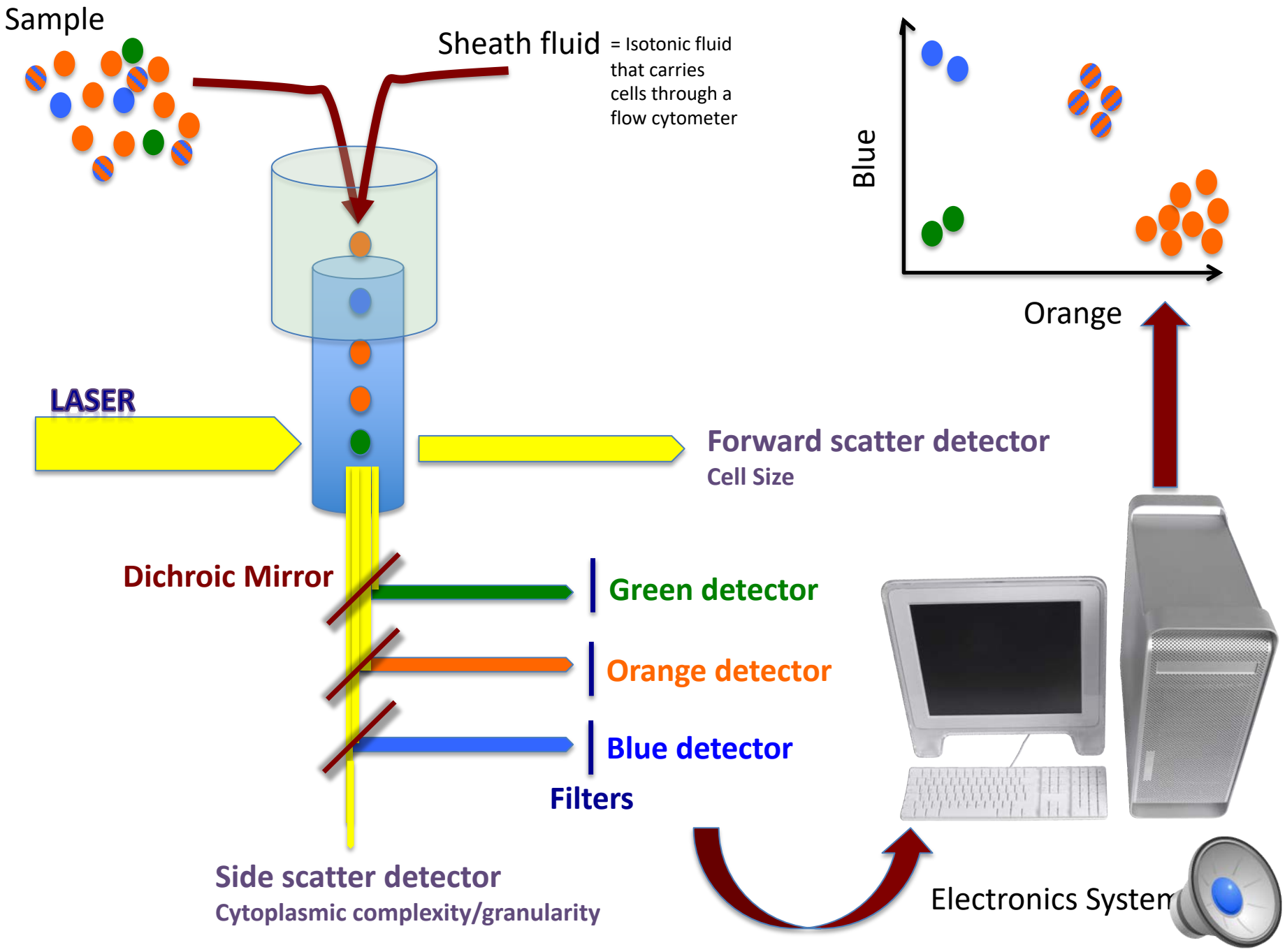
B-cell markers
CD19, CD20, kappa, lambda

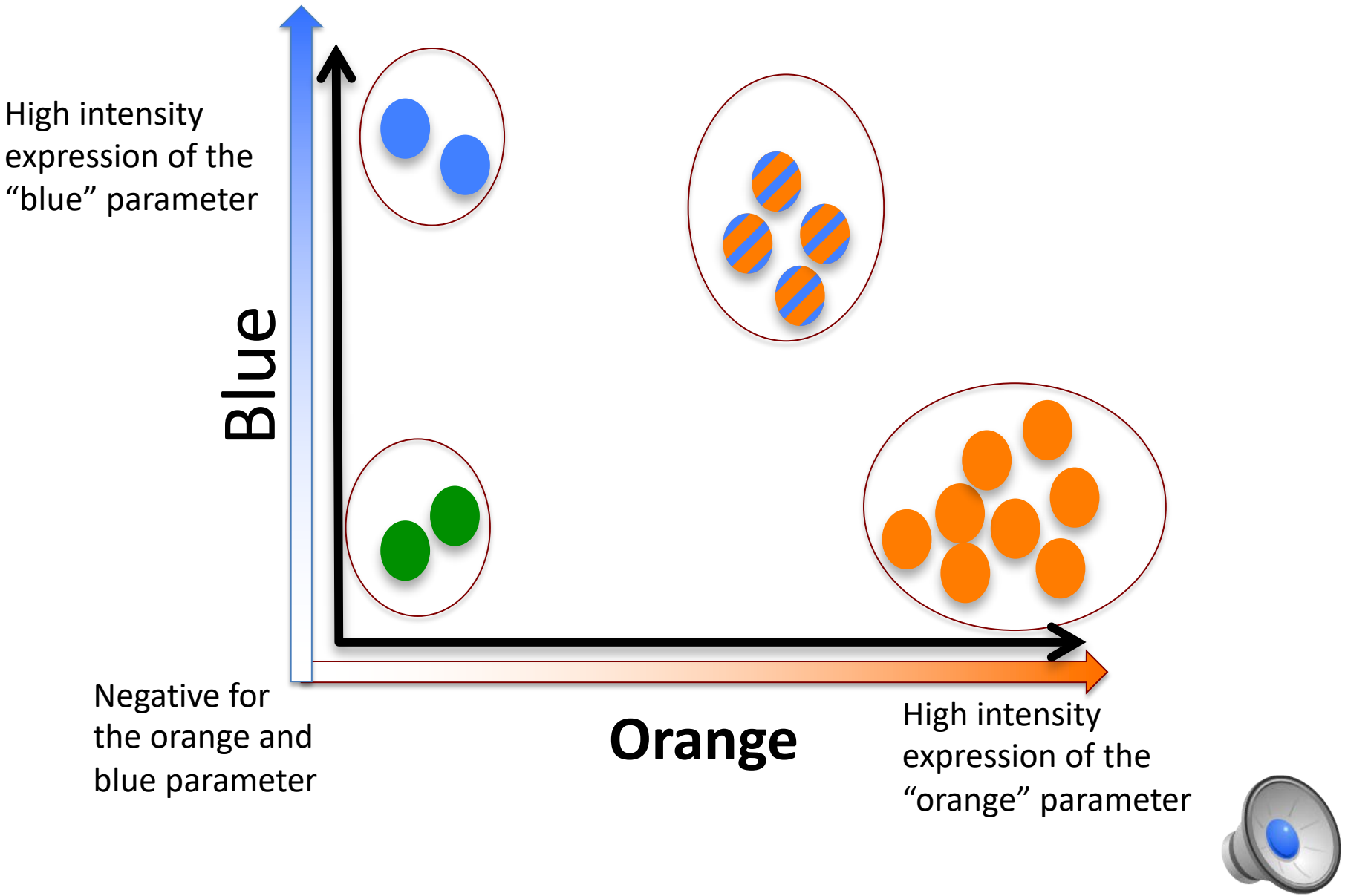
T-cell markers
CD2, CD3, CD5, CD7, CD4, CD8

NK cell marker
CD16, CD56

Other, not lineage specific
CD38, HLA-DR

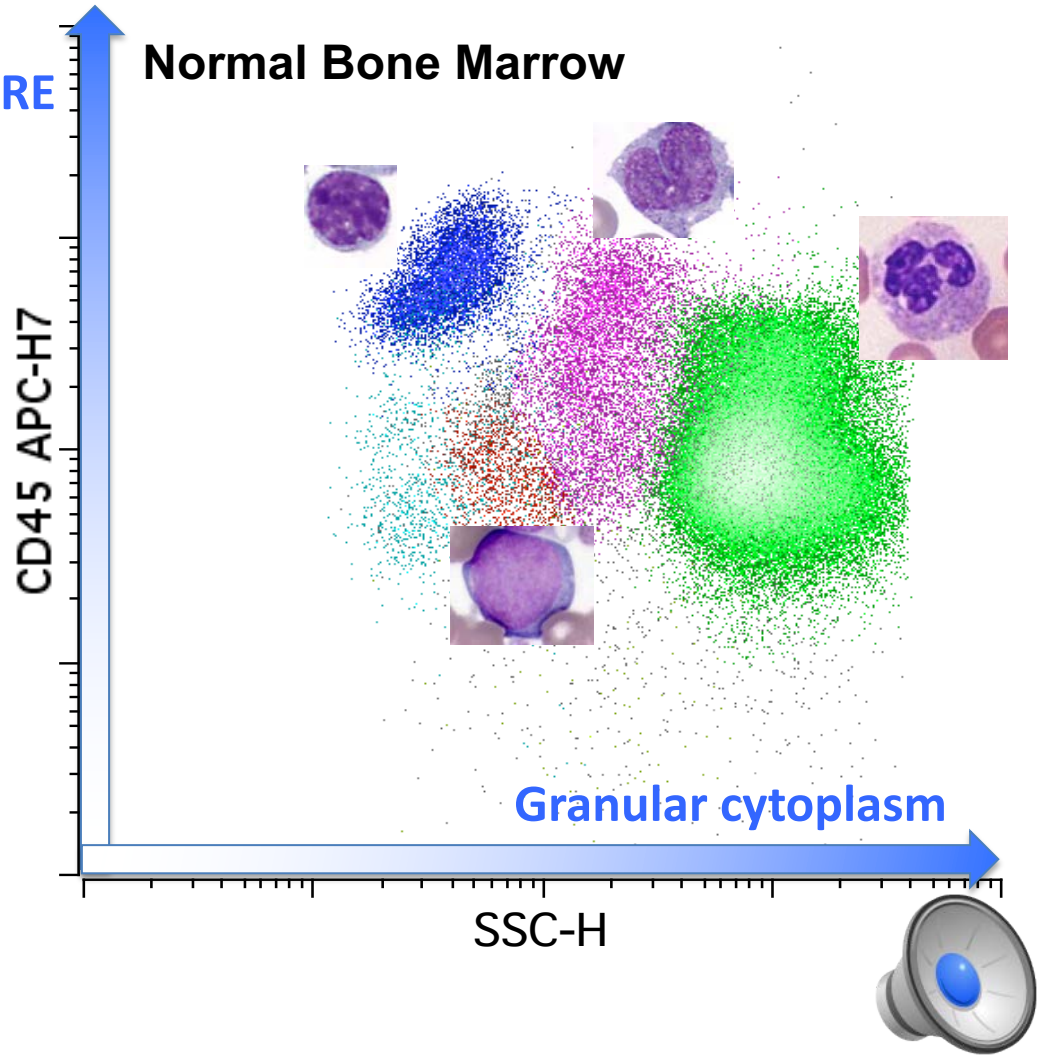

Unstained Sample
Stained Sample
SSC
FSC
Orange signal
No blue signal
No green signal


Sample
Sheath fluid
= Isotonic fluid that carries cells through a flow cytometer
Blue
Orange
LASER
Forward scatter detector
Cell Size
Dichroic Mirror
Green detector
Orange detector
Blue detector
Filters
Side scatter detector
Cytoplasmic complexity/granularity
Electronics System


High intensity expression of the "blue" parameter
Blue
Negative for the orange and blue parameter
Orange
High intensity expression of the "orange" parameter

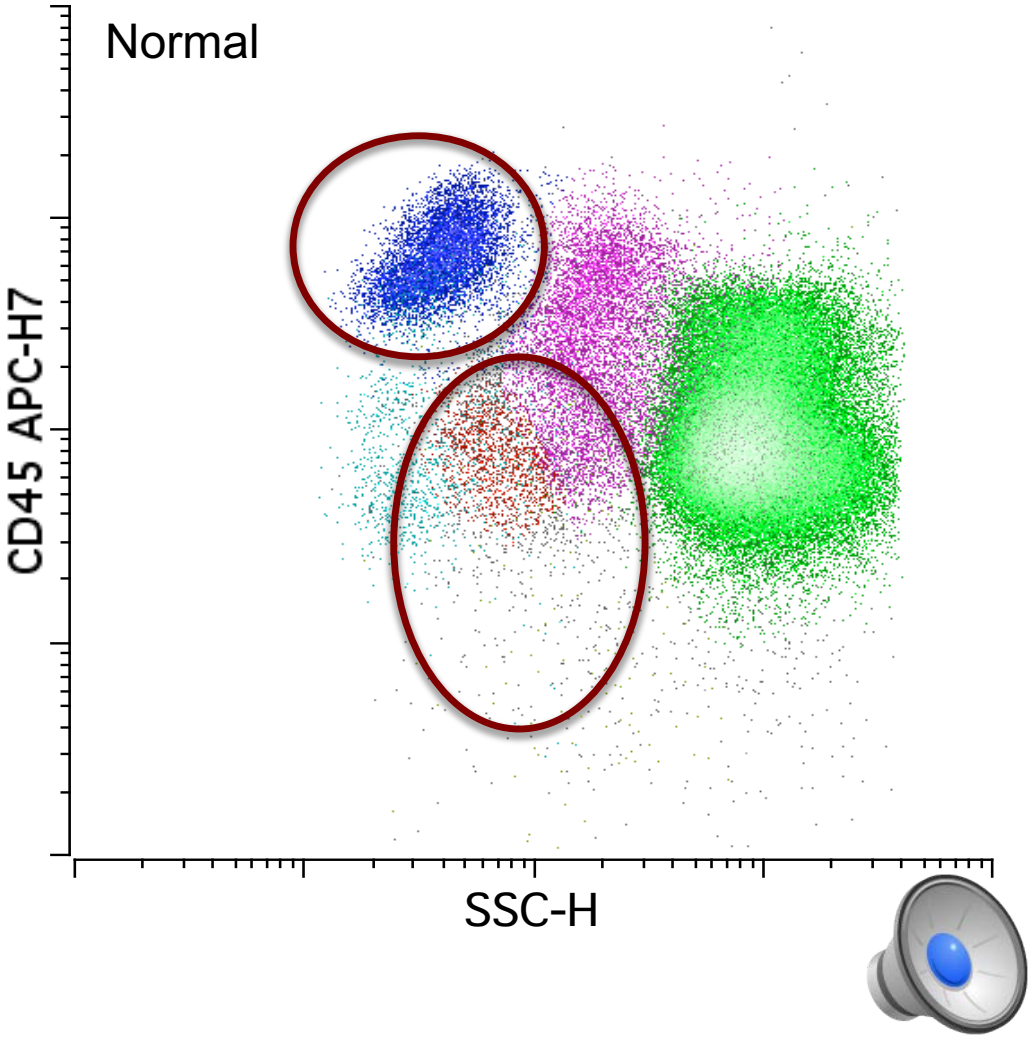
How does flow cytometry work in practice?

- Many samples are complex and contain different cell types
- We start by identifying a population of interest
- CD45 versus SSC analysis is a good first step for population identification
- To the left is an example of a bone marrow aspirate

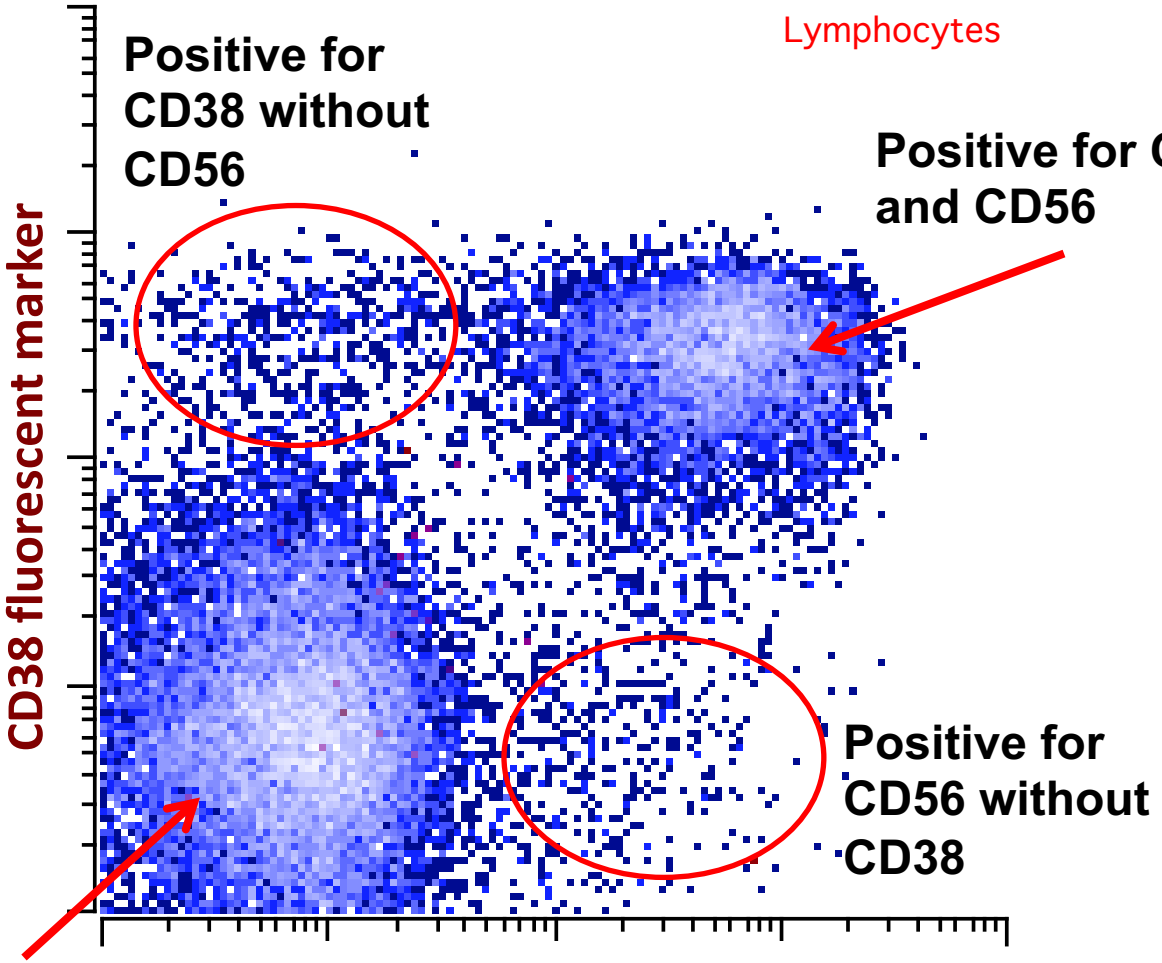


How does flow cytometry work in practice?

- Once you identify a population of interest, to draw a gate around it and look at antigen expression specifically in that population
  - If you are concerned about acute leukemia, you would gate the blasts
  - If you are concerned about lymphoma, you would gate the lymphocytes



How do you read a flow cytometry dot plot?



Take home points

- Flow cytometry is a tool used to characterize the immunophenotype (pattern of antigen expression) of hematopoietic cells
- Performed on fresh specimens
- Critical part of diagnosis and classification of hematopoietic neoplasms