Course of Andrology I

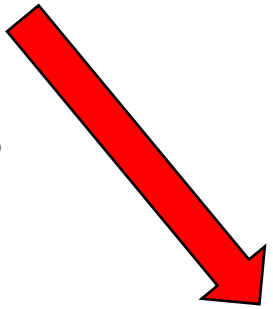
PRINCIPLE OF FLOW CYTOMETRY

Prof. Alberto Contri

DEFINITIONS

FLOW CYTOMETRY

WHAT SUGGESTS THAT TERM?



MEASUREMENT OF THE CELLULAR ATTRIBUTES

FLOW – DYNAMIC MOVEMENT OF THE CELLS THROUGH THE SYSTEM

DEFINITIONS

FLOW CYTOMETRY

AT THE ORIGIN: MEASUREMENT OF THE CELL SIZE

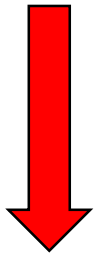
TODAY: MEASUREMENT OF 14 PARAMETERS SIMULTANEOUSLY

DEFINITIONS

FLOW CYTOMETRY

MEASURE THE OPTICAL AND FLUORESCENCE CHARACTERISTICS OF ELEMENTS IN

A FLUID STREAM WHEN THEY PASS THROUGH A LIGHT SOURCE



CELLS

MICROORGANISMS

NULEUI

SUBCELLULAR OR SMALL PARTICLES

FLOW CYTOMETRY

2 TYPES

NON SORTING FC

MEASURE THE LIGHT AND FLUORESCENCE EMISSION OF THE EVENTS

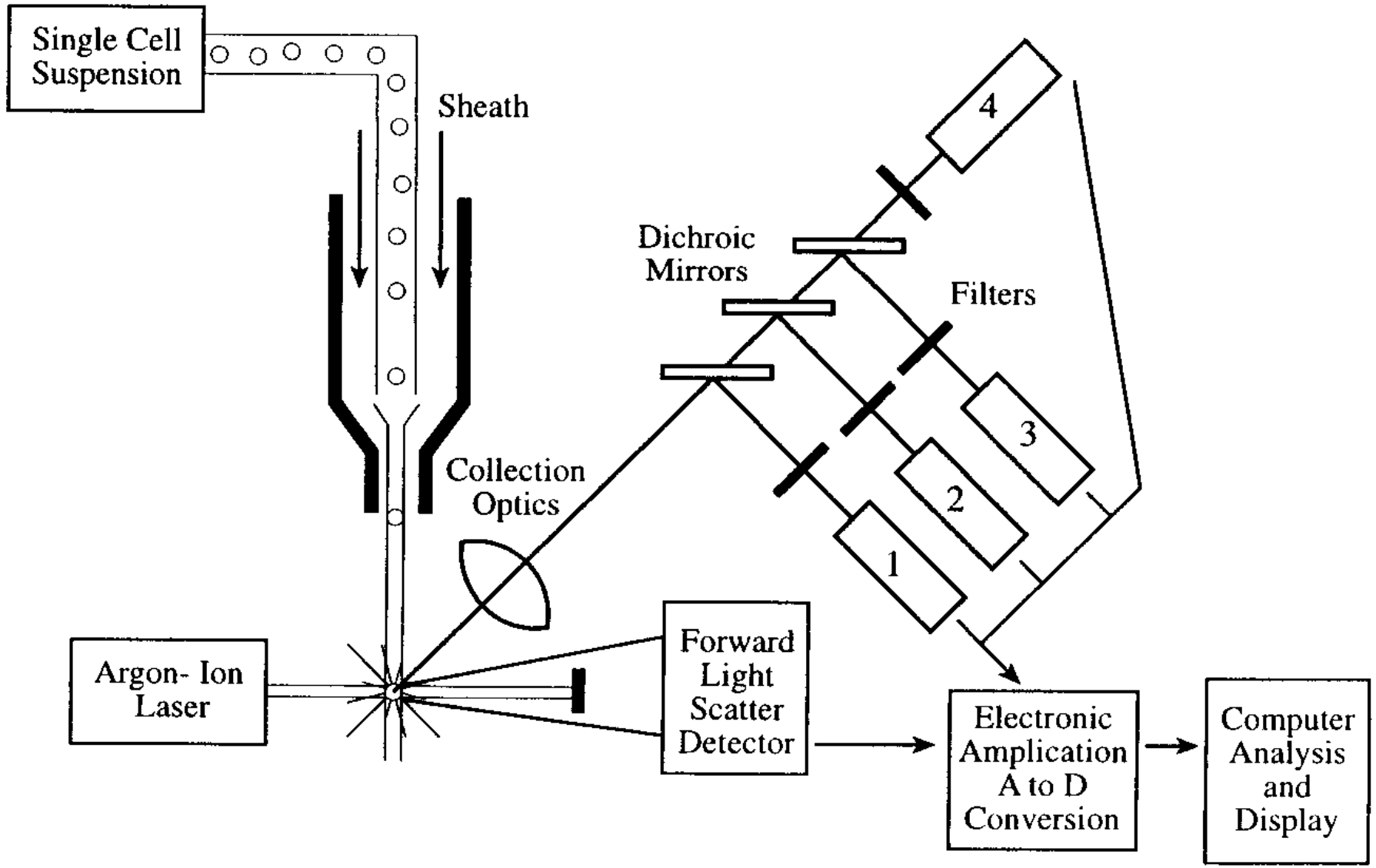
SORTING FC

FLUORESCENCE ACTIVATED FLOW CYTOMETRY (FACS)

SEPARATION OF THE EVENTS IN DIFFERETN SUBPOPULATIONS

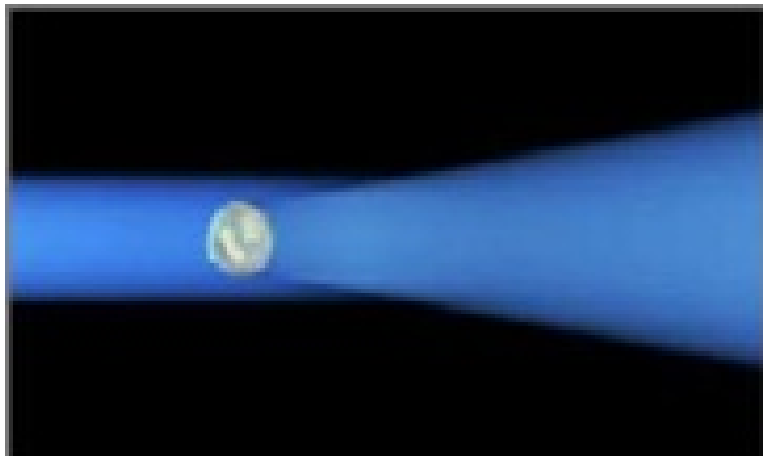
COMPONENTS

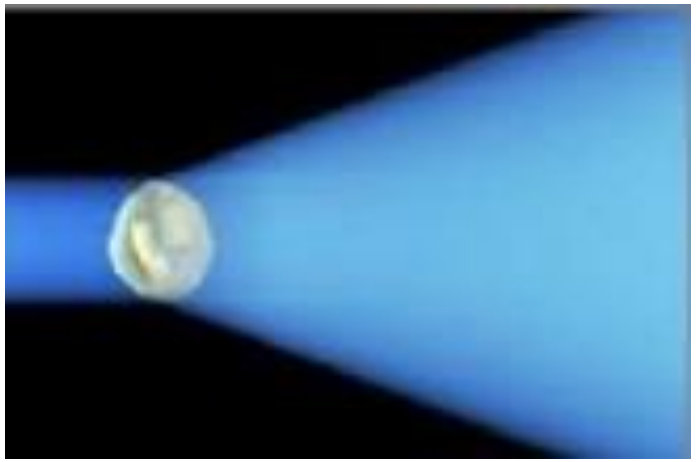
FLUIDICS	MOVEMENT OF THE ELEMENTS FLOW OF THE ELEMENTS TO THE LIGHT SOURCE
OPTICS	EXCITATION AND COLLECTION FOCUSES THE LIGHT ON THE ELEMENTS AND TRANSMIT THE LIGHT SCATTER/FLUORESCENCE EMISSION TO THE ELECTRONICS
ELECTRONICS	DETECTION AND ELABORATION DETECT LIGHT/FLUORESCENCE ATTRIBUTE AND PROPORTIONALLY REPORTED ON A PROCESSOR


Single Cell Suspension
Sheath
Dichroic Mirrors
Filters
4
3
2
1
Collection Optics
Argon- Ion Laser
Forward Light Scatter Detector
Electronic Amplication A to D Conversion
Computer Analysis and Display

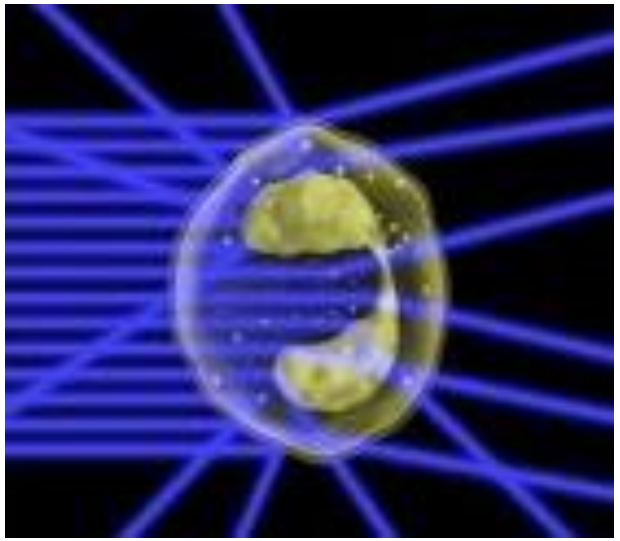
MORPHOLOGICAL PARAMETERS

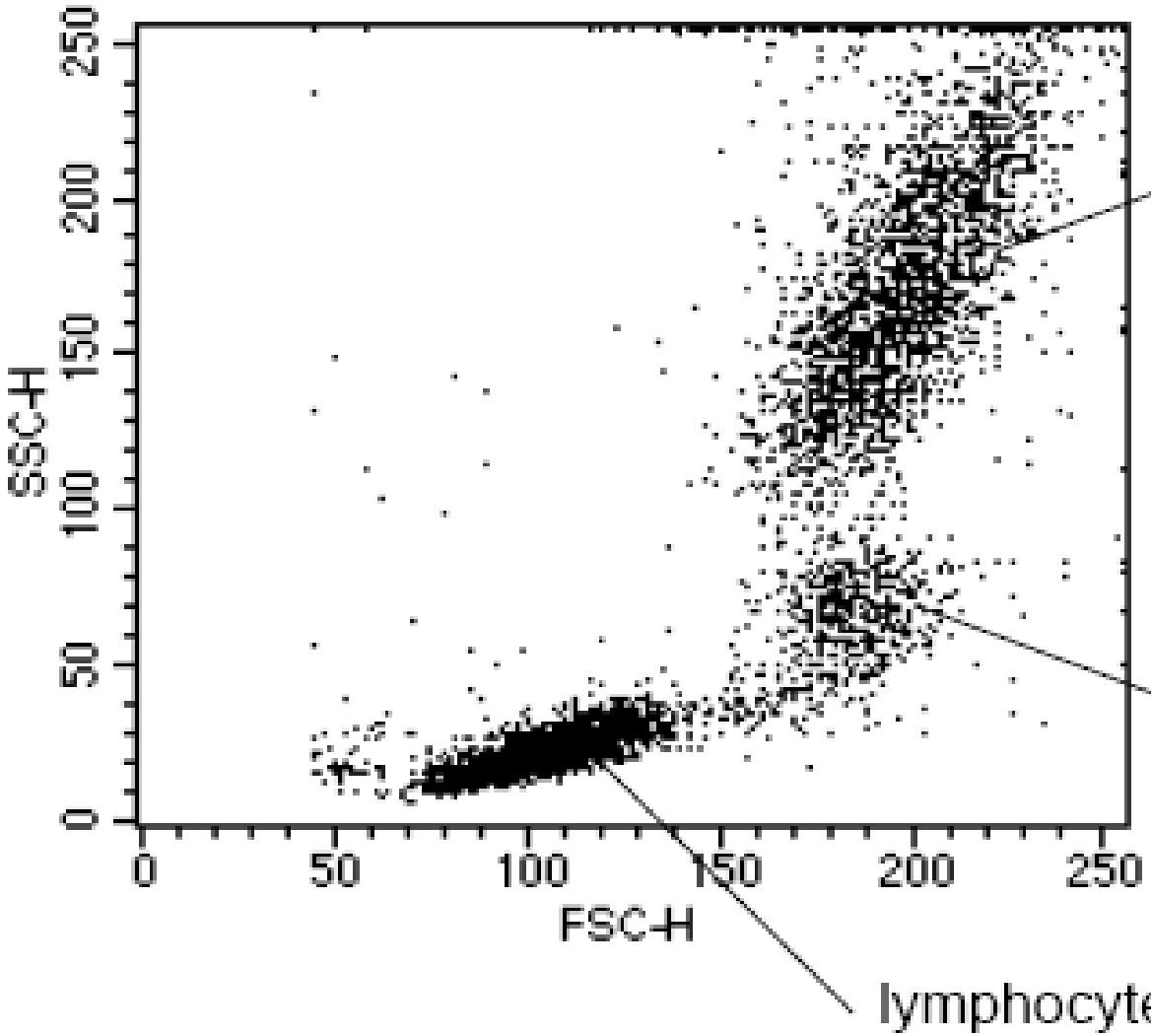
Forward scatter





Side scatter





lysed whole blood

FLUORESCENCE ATTRIBUTES

FLUORESCENCE

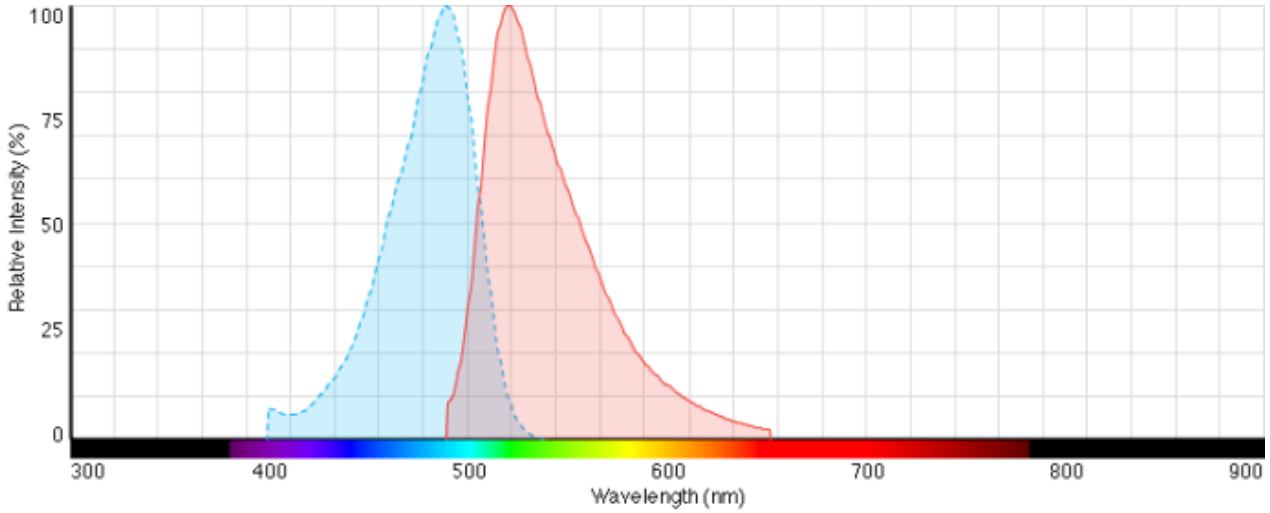
Compound absorbs the light at a specific wavelength

Excitation of the compound – electrons at higher energy level

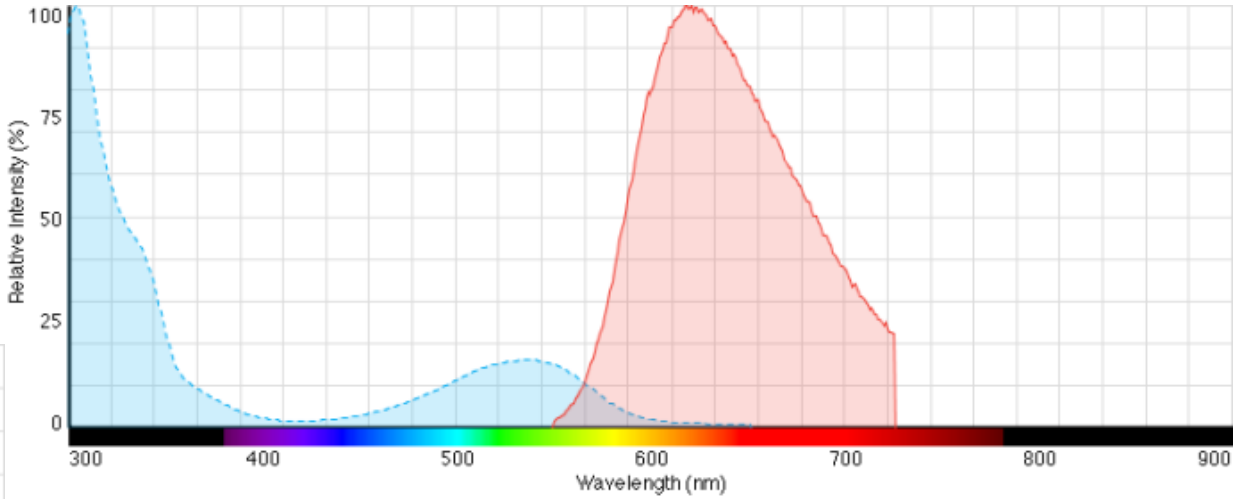
Excitated eletrons emit light photons at specific wavelengts - spectrum

FLUORESCENCE ATTRIBUTES

FLUORESCENCE



SYBR-14



Propidium iodide

FLUORESCENCE ATTRIBUTES

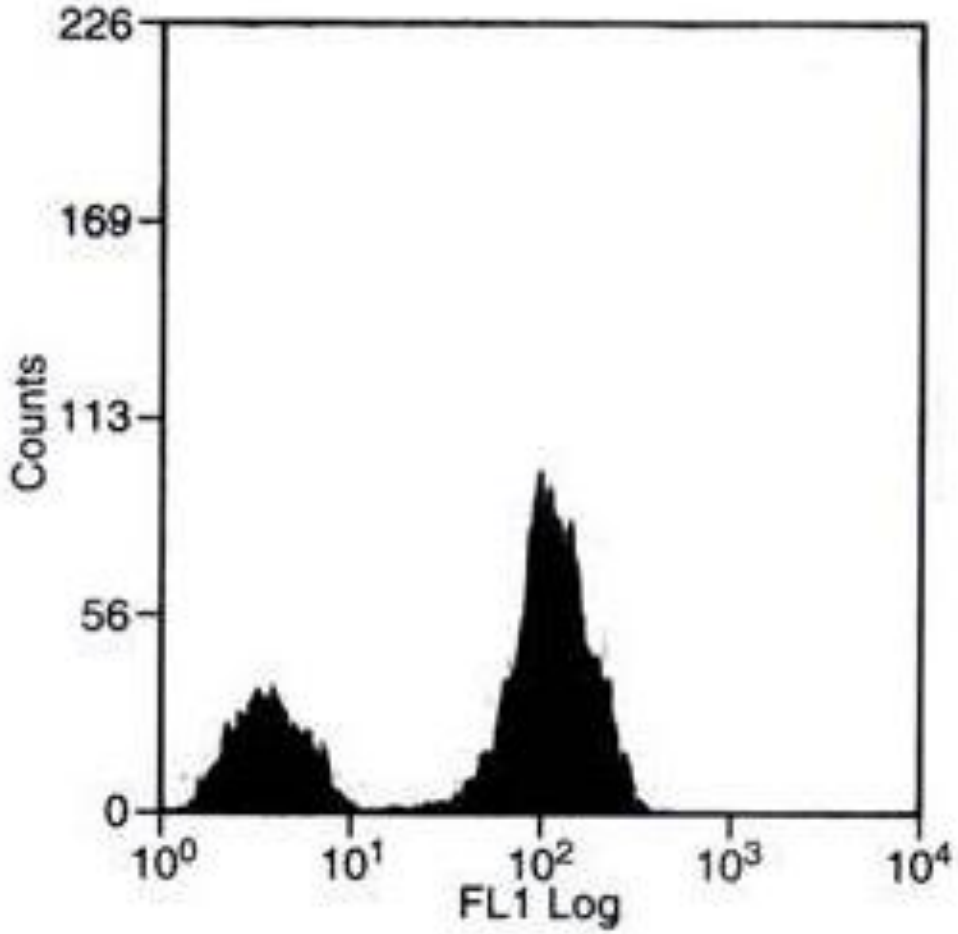
ELECTRONICS

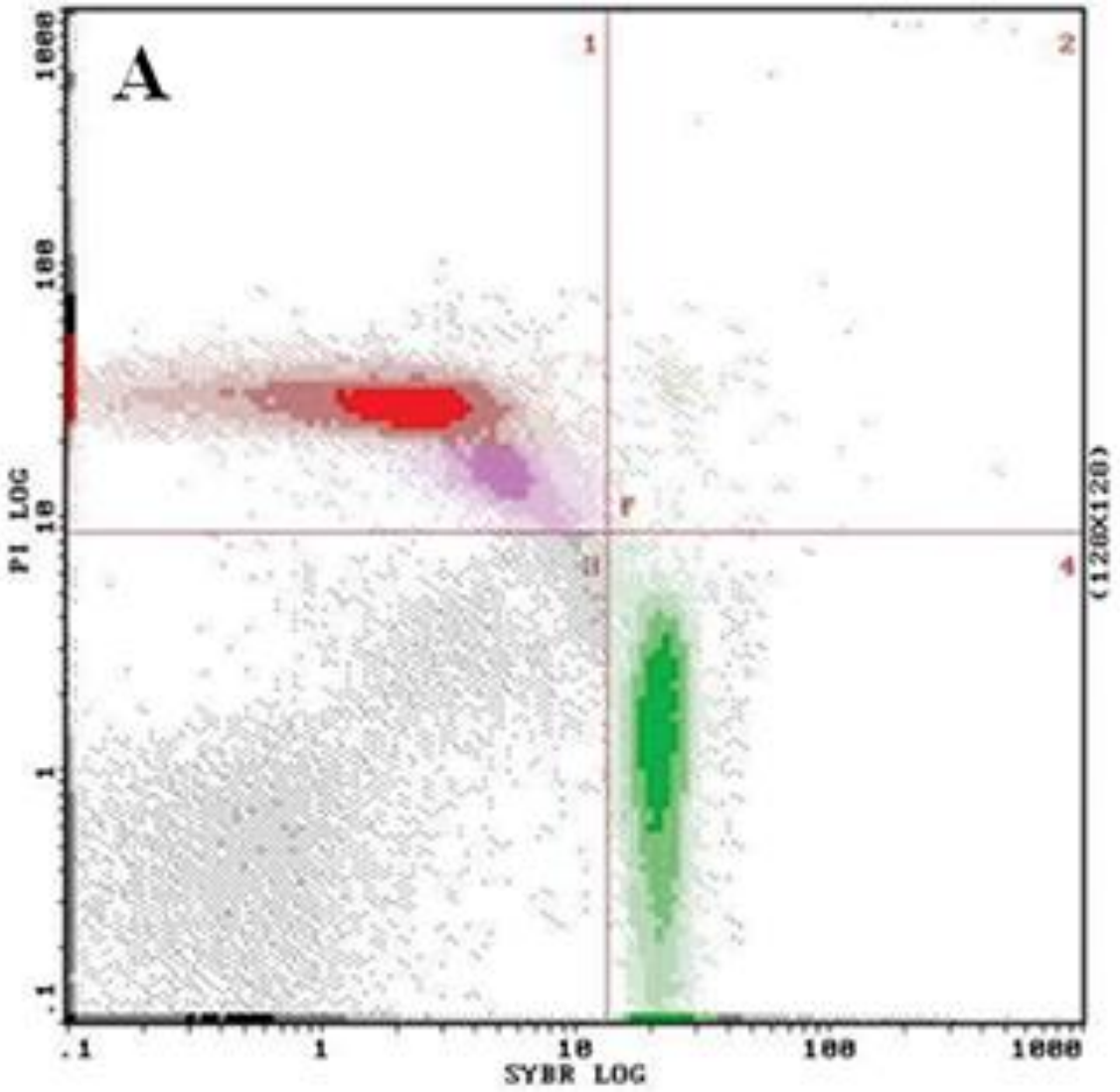
Detection of specific wavelength by different detectors

Photodiodes – efficiency of 50%

Photomultipliers – efficiency of 2000%

PLOT INTERPRETATION





SAMPLE PREPARATION

Prepare the sample according to the protocol

relevant factors

TIME

TEMPERATURE

ANALYSIS PREPARATION

Calibration of the machine

- Periodical calibration of the lasers
- Use unstained sample for the fluorescence detector calibration
- Calibrate each fluorescence in multi-parametric analysis
- Run all the samples

ANALYSIS OF THE SAMPLE

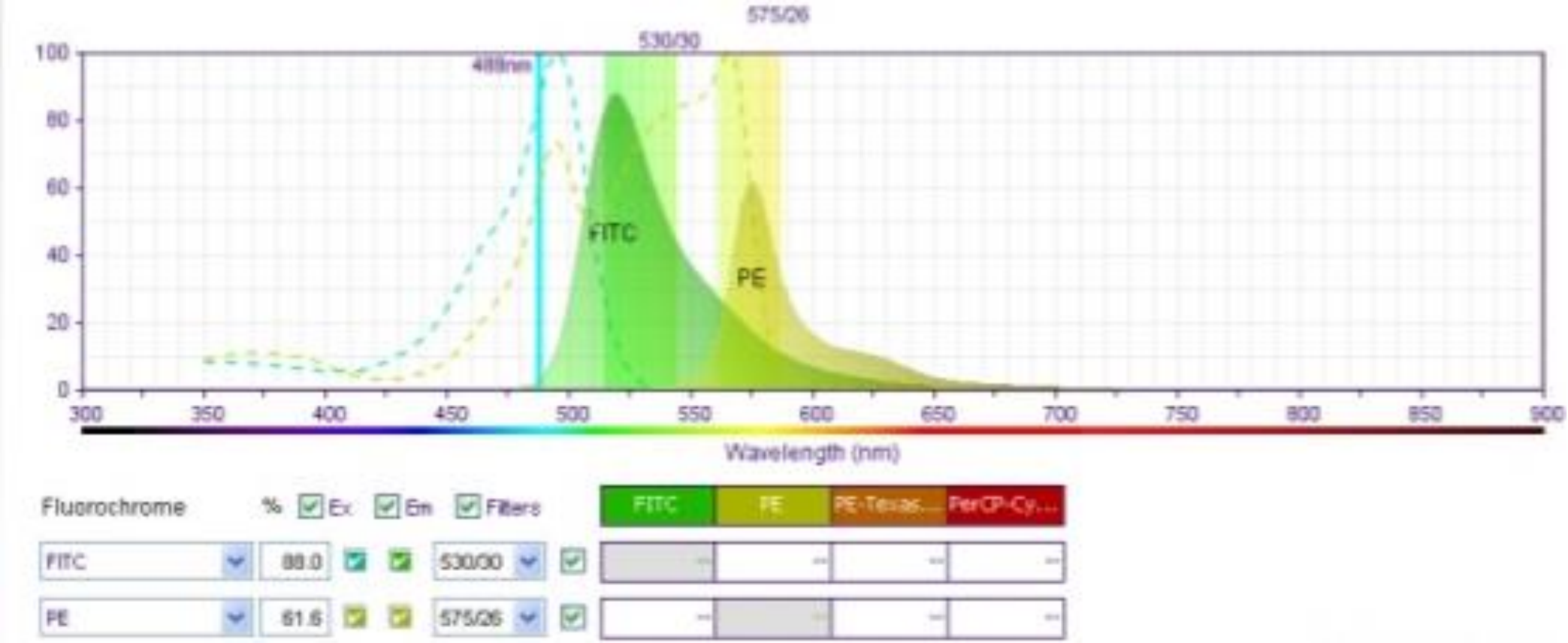
Fluorescence compensation

Required in multiparametric anaysis

Remove the interference of the emission spectra from the different fluorochromes

ANALYSIS OF THE SAMPLE

Fluorescence compensation



ANALYSIS OF THE SAMPLE

Fluorescence compensation

Run each fluorochrome alone ad adjust detectors

Run fluorochromes simultaneously