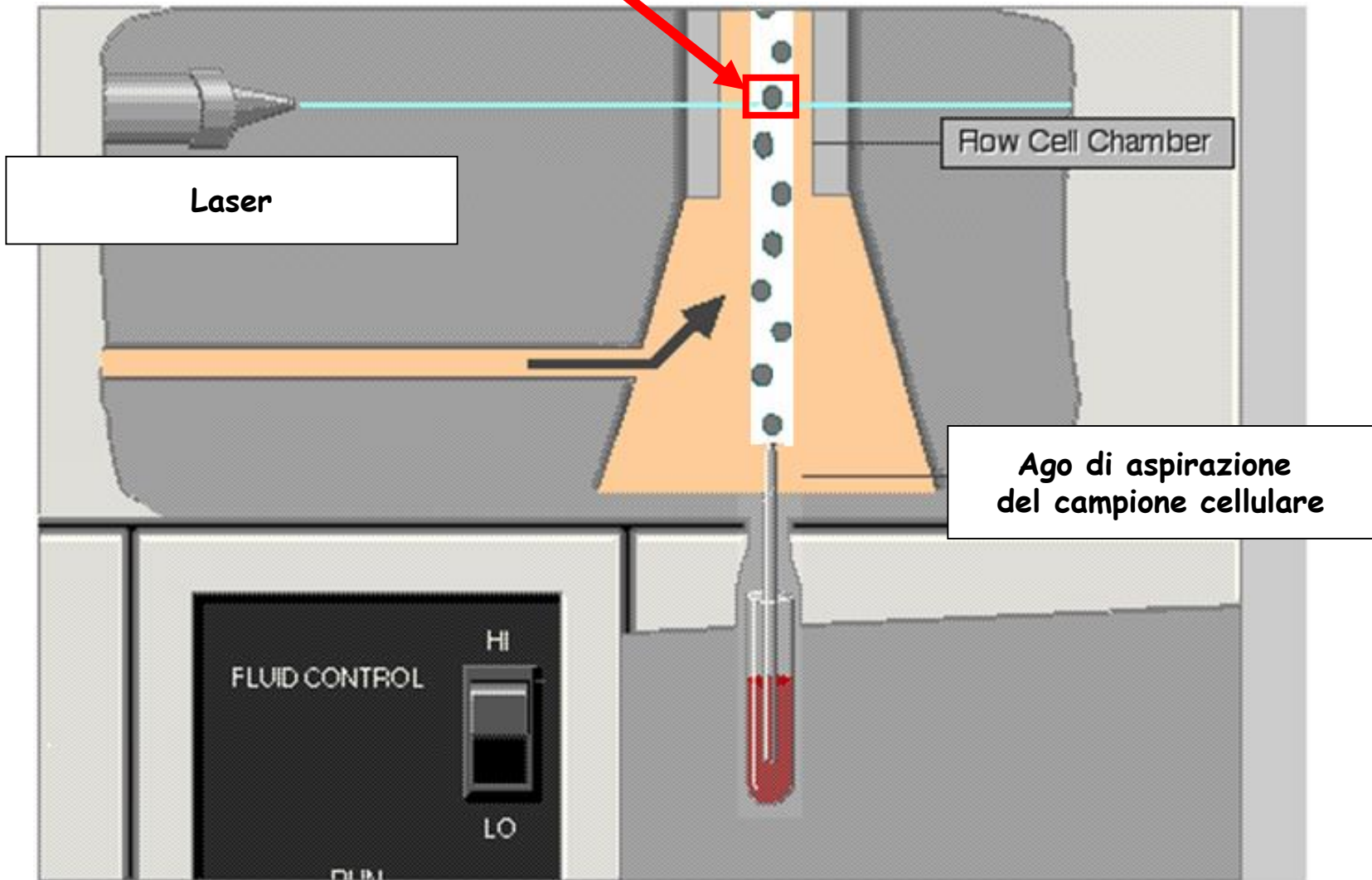


DENOThe Meeting

1 Ottobre 2019

***«Caratterizzazione e isolamento di subset cellulari mediante
analisi citofluorimetrica multiparametrica»***

Punto di intercettazione
della cellula



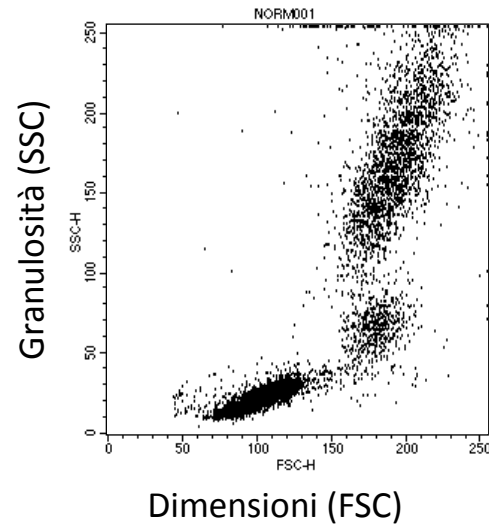
Flow Cell Chamber

Laser

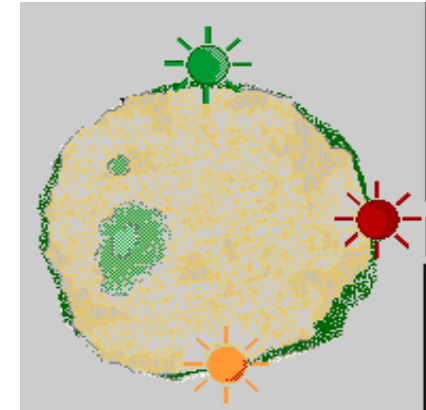
Ago di aspirazione
del campione cellulare

FLUID CONTROL
HI
LO

Parametri intrinseci (fisici)



Parametri estrinseci



Comprendono le caratteristiche fenotipiche e funzionali delle cellule e vengono messe in evidenza attraverso l'utilizzo di fluorocromi.

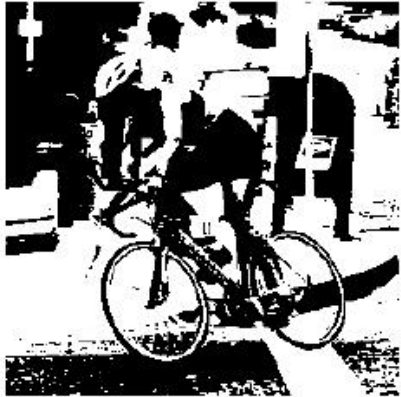
I fluorocromi:

1. Possono legarsi spontaneamente a specifiche componenti cellulari, come le proteine o il DNA.
2. Possono essere coniugati ad anticorpi monoclonali, per l'individuazione di specifiche molecole di membrana o intracellulari.

Più colori

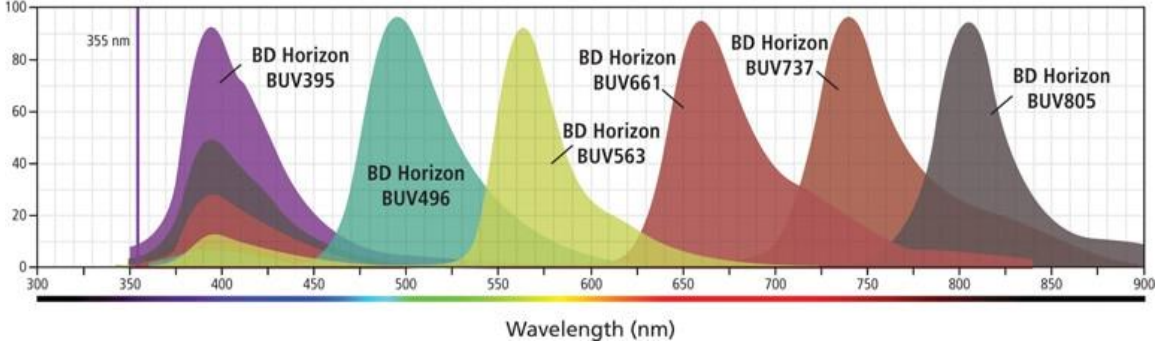


Maggiori informazioni

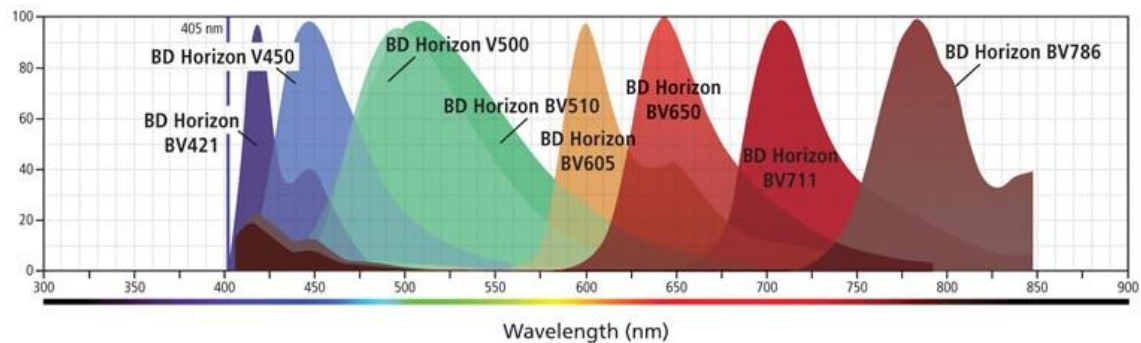


Più è alto il numero di fluorocromi utilizzabili contemporaneamente e maggiore è il numero di informazioni che possiamo ottenere su singola cellula

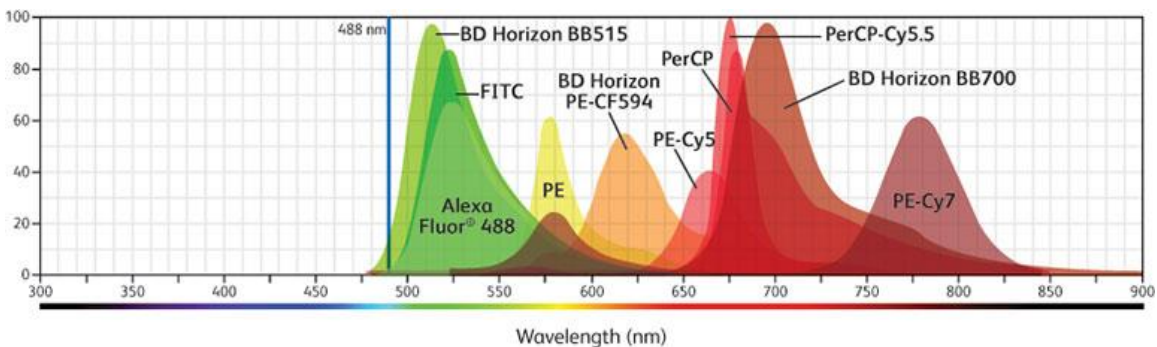
UV



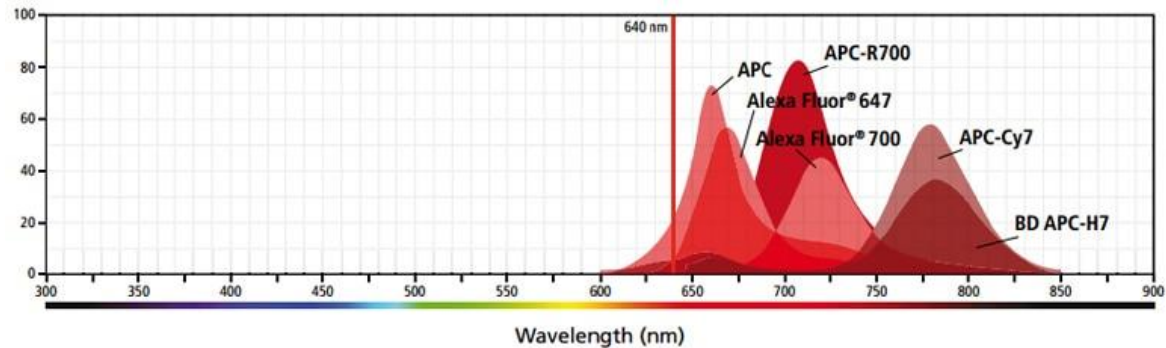
Violet



Blue



Red



Citofluorimetri della UR1



UV

488 nm (blue Laser)

633 nm (red laser)

405 nm (violet laser)

8 colori



488 nm (blue Laser)

633 nm (red laser)

405 nm (violet laser)

11 colori



488 nm (blue Laser)

633 nm (red laser)

405 nm (violet laser)

8 colori

Applicazioni della citofluorimetria

-Immunophenotyping

-Cell cycle analysis

-Cell proliferation

-Apoptosis

-Cell signaling

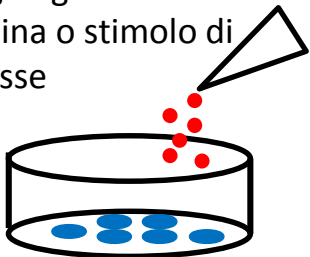
-Rare events

-Cell sorting

...

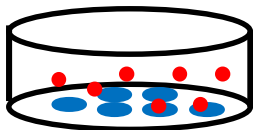
Cell signaling: TCR

1. Aggiungere in vitro citochina o stimolo di interesse



2. Time course

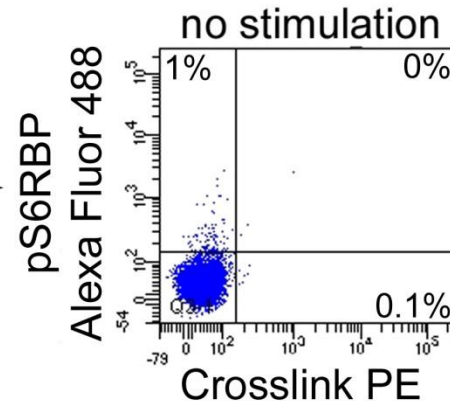
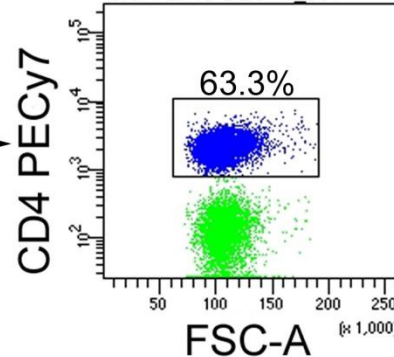
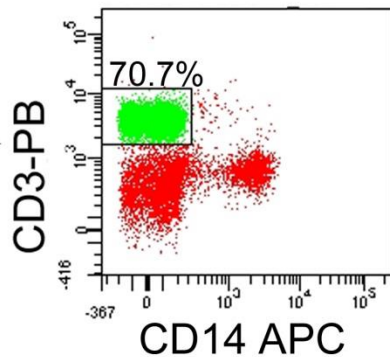
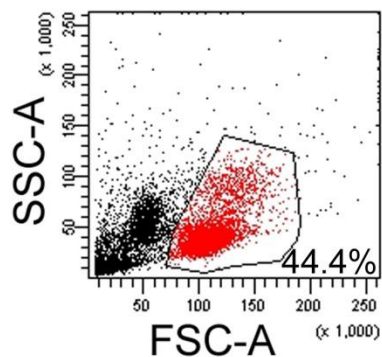
10' 20' 30'...



3. Cell fixation

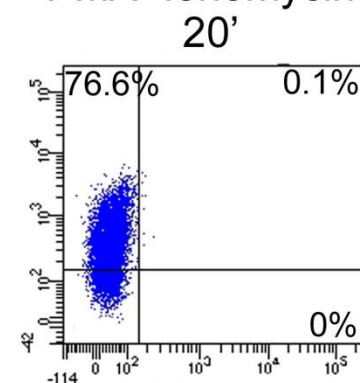
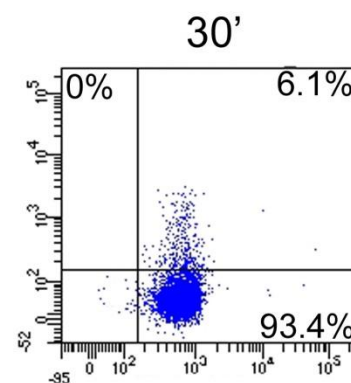
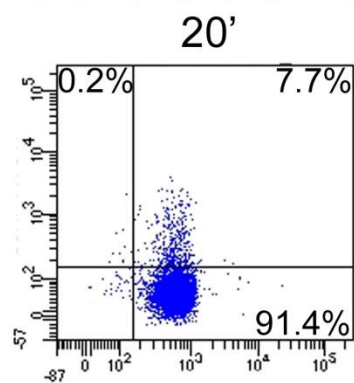
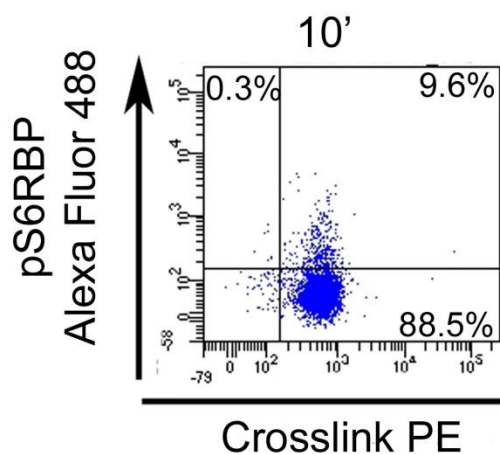
4. Cell permeabilization

5. Staining

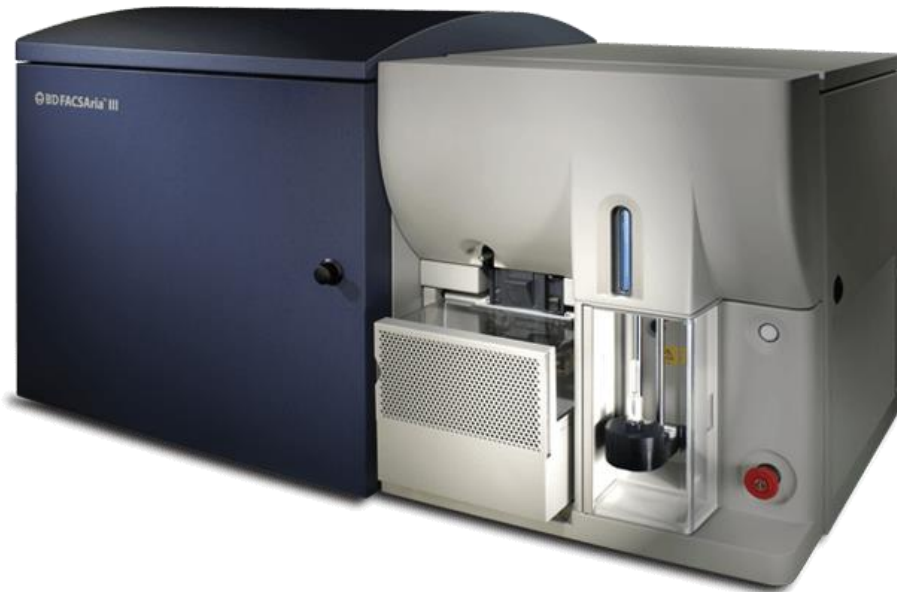


anti-CD3+anti-CD28

PMA+Ionomycin



Cell sorting



UV

488 nm (blue Laser)

633 nm (red laser)

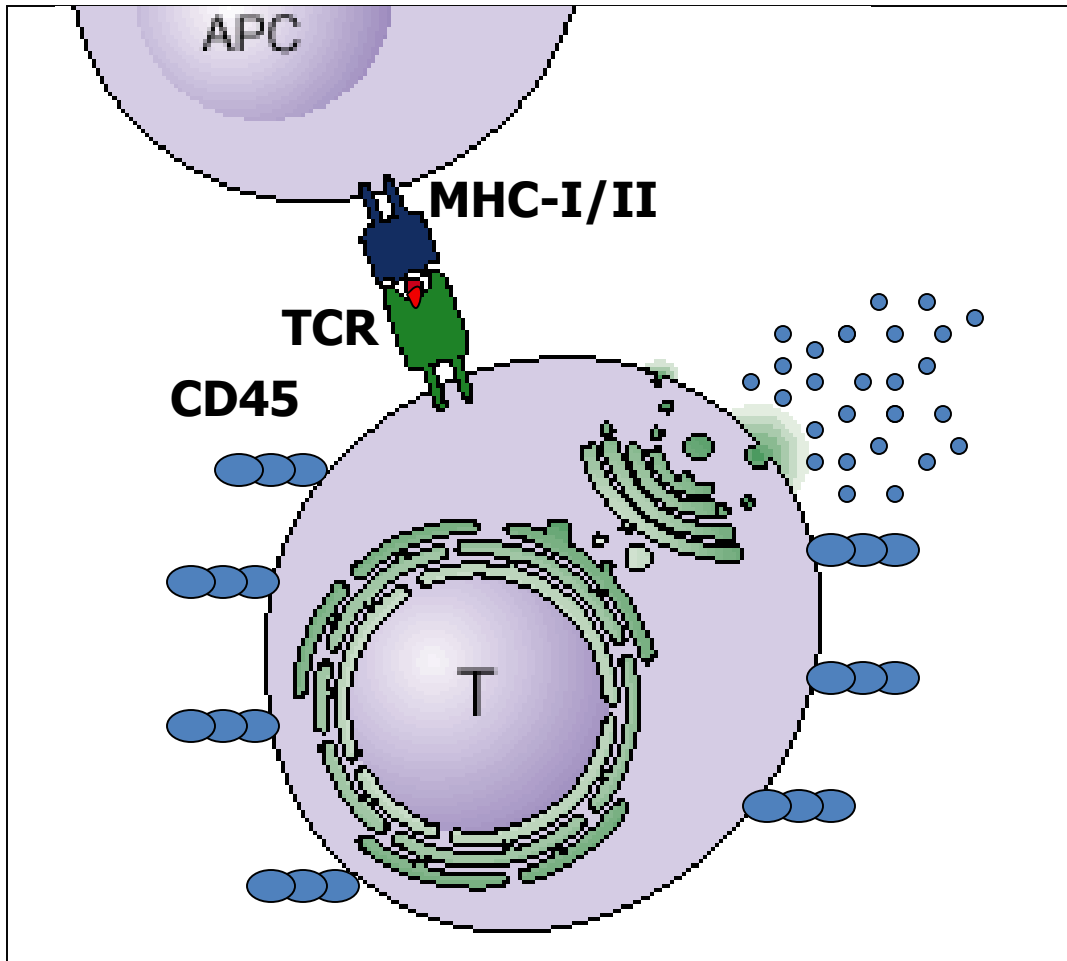
405 nm (violet laser)

8 colori

-Fino a 4 differenti popolazioni di cellule vive recuperate (bulk sorting) in provetta

-Sorting in piastra (bulk o single cell)

Isolamento di cellule vive in base alla loro capacità di produrre citochine: Cytokine secretion assay (1)

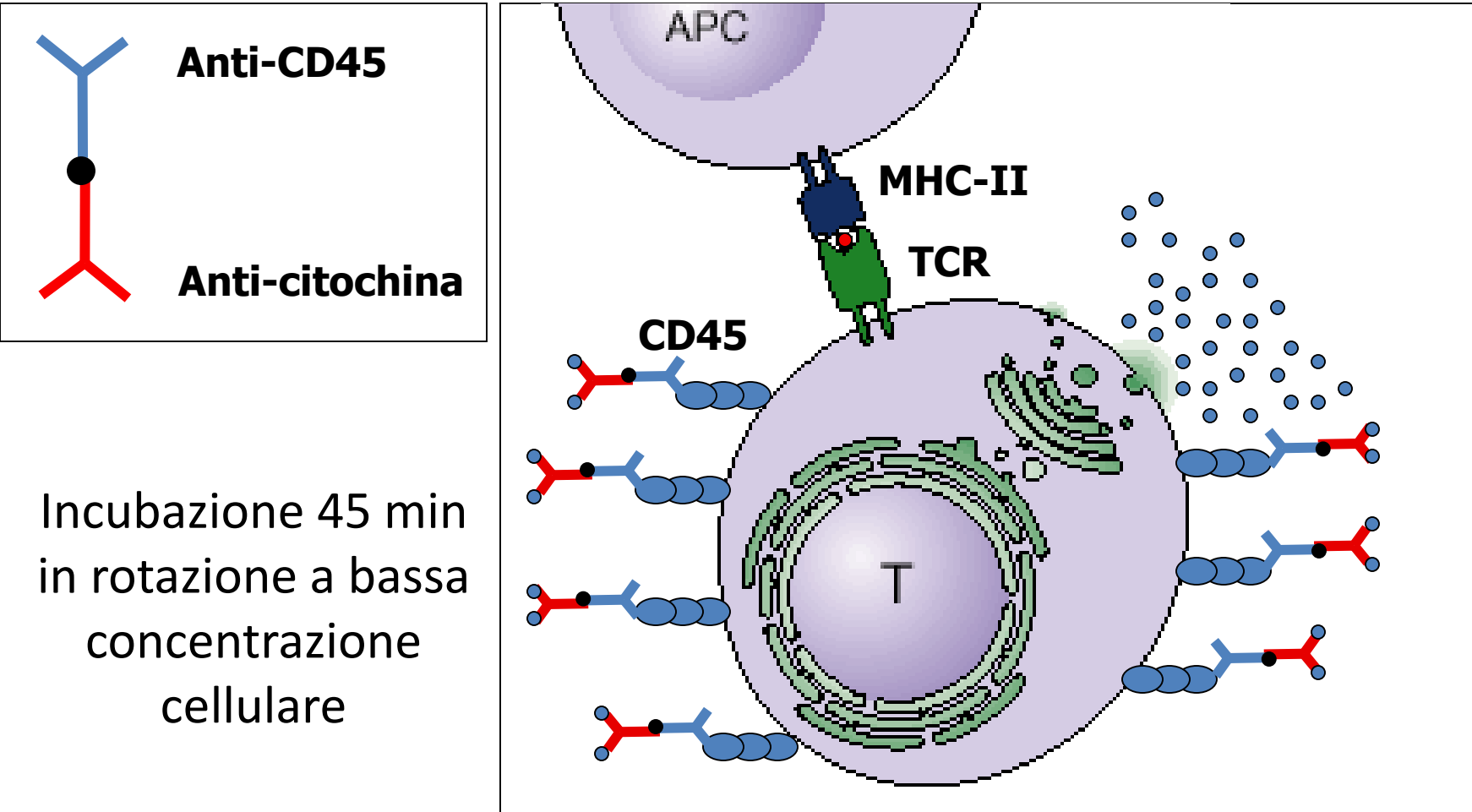


I tempi di stimolazione del campione sono:

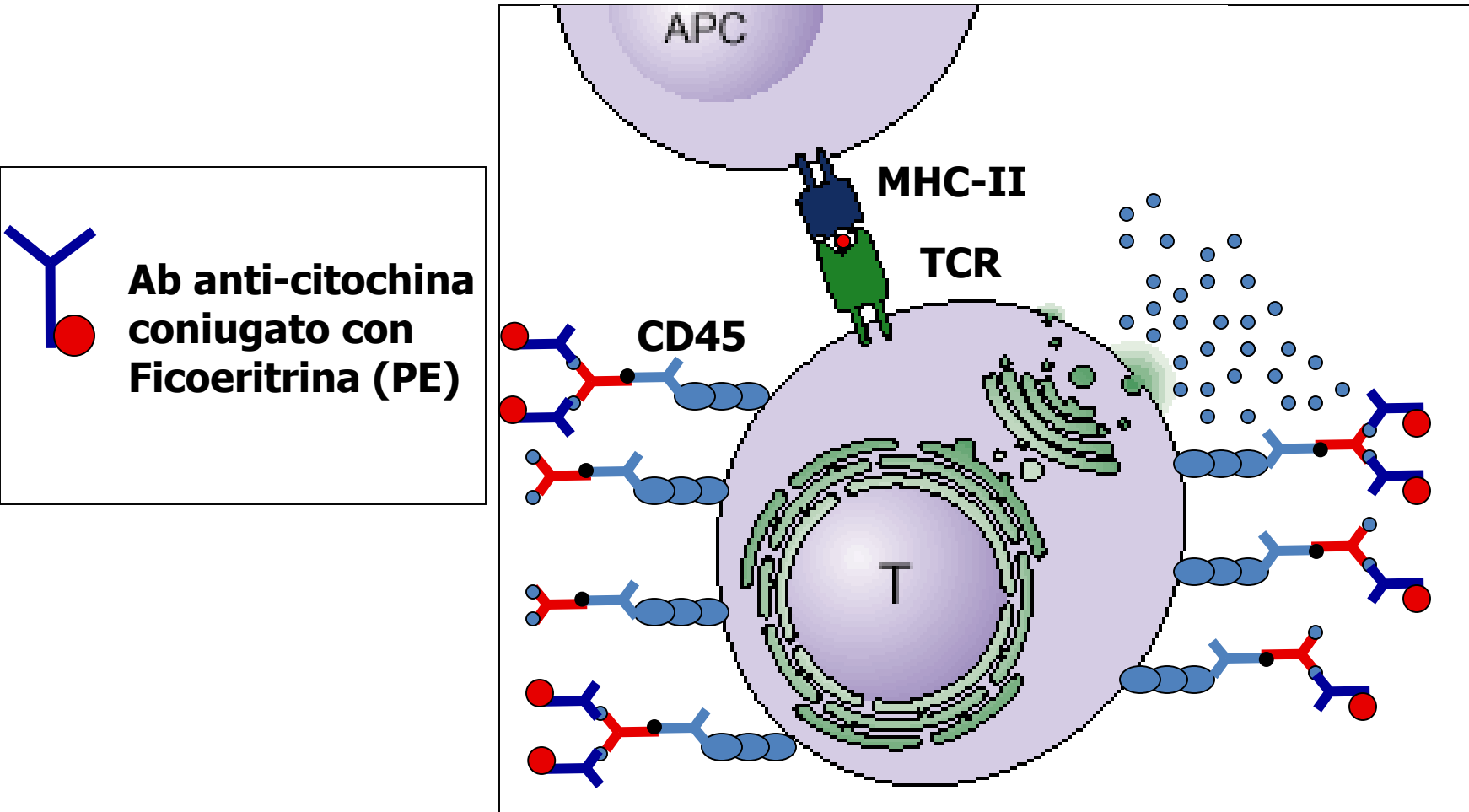
3-16 ore con peptidi

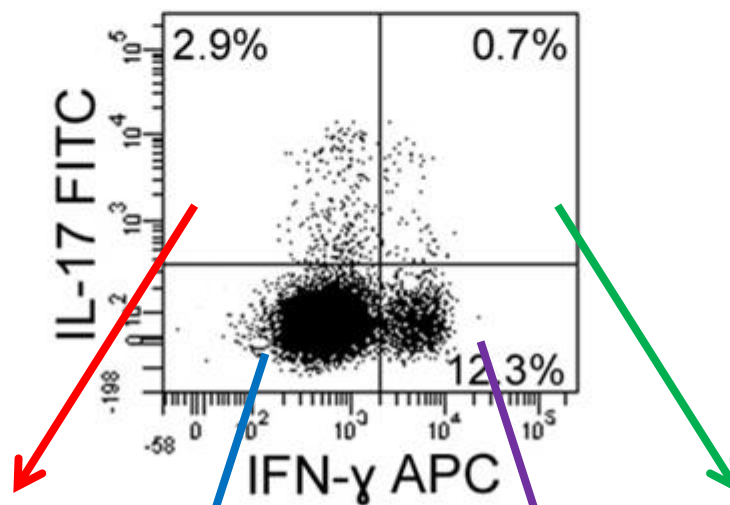
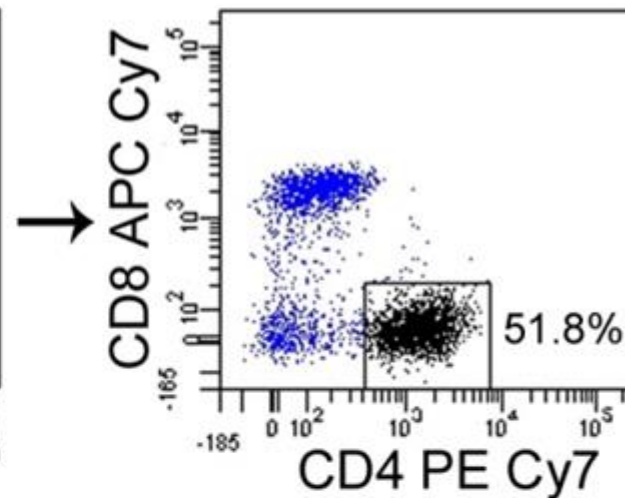
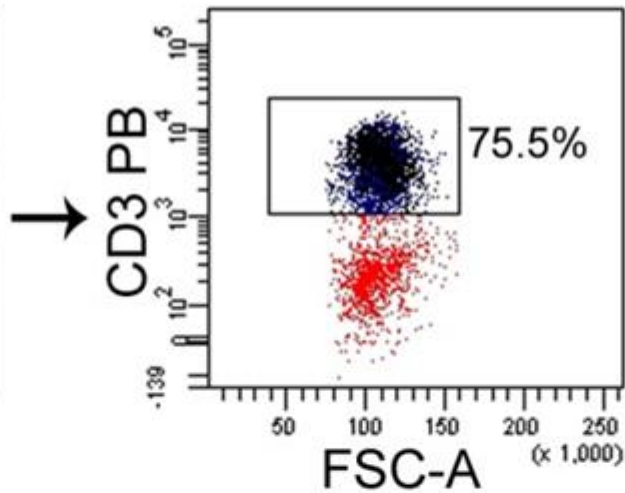
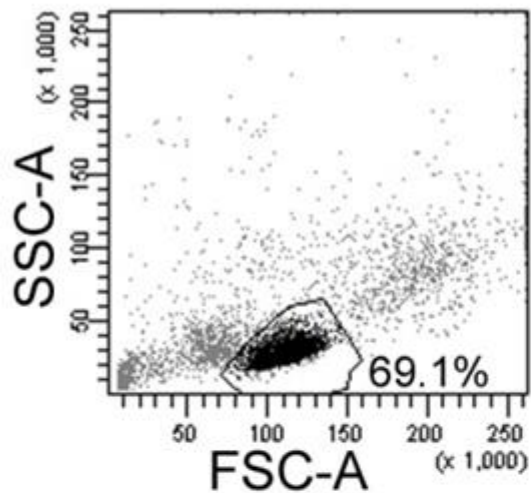
6-16 ore con proteina

Isolamento di cellule vive in base alla loro capacità di produrre citochine: Cytokine secretion assay (2)



Isolamento di cellule vive in base alla loro capacità di produrre citochine: Cytokine secretion assay (3)





IL17+
IFN-g-

IL17- IFN-g+

IL17+ IFN-g+

IL17- IFN-g-