

A flow cytometria alkalmazása a  
hematológiai betegségek  
diagnosztikájában és terápiájában

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PTE, KK, Pathologiai Intézet

# A flow cytometria alapjai

## **Definíció:**

A flow cytometria - **FCM** - folyadékban áramló partikulumok optikai tulajdonságainak vizsgálatára alkalmas módszer.

## **Működése:**

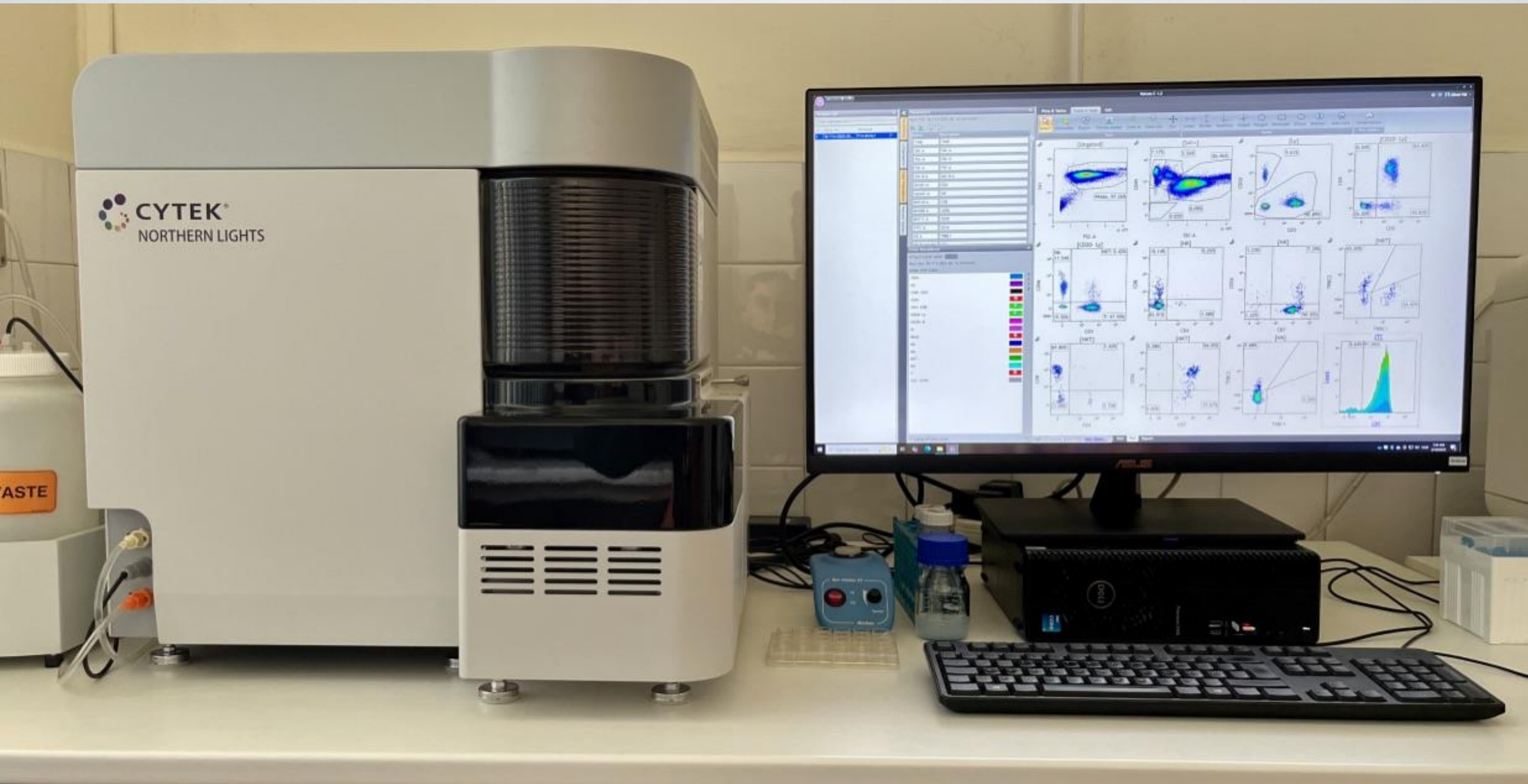
1. Sejtszuszpenziót injektálnak egy köpenyfolyadékba, ahol a sejtek egyenként haladnak el, egy az áramlásra merőleges monokromatikus fényforrás előtt.
2. A sejtáram és a gerjesztő fénysugár metszéspontjában sejtenként, egyesével fényszórási és fluoreszcens jelek detektálhatók.

# A flow cytometer működése

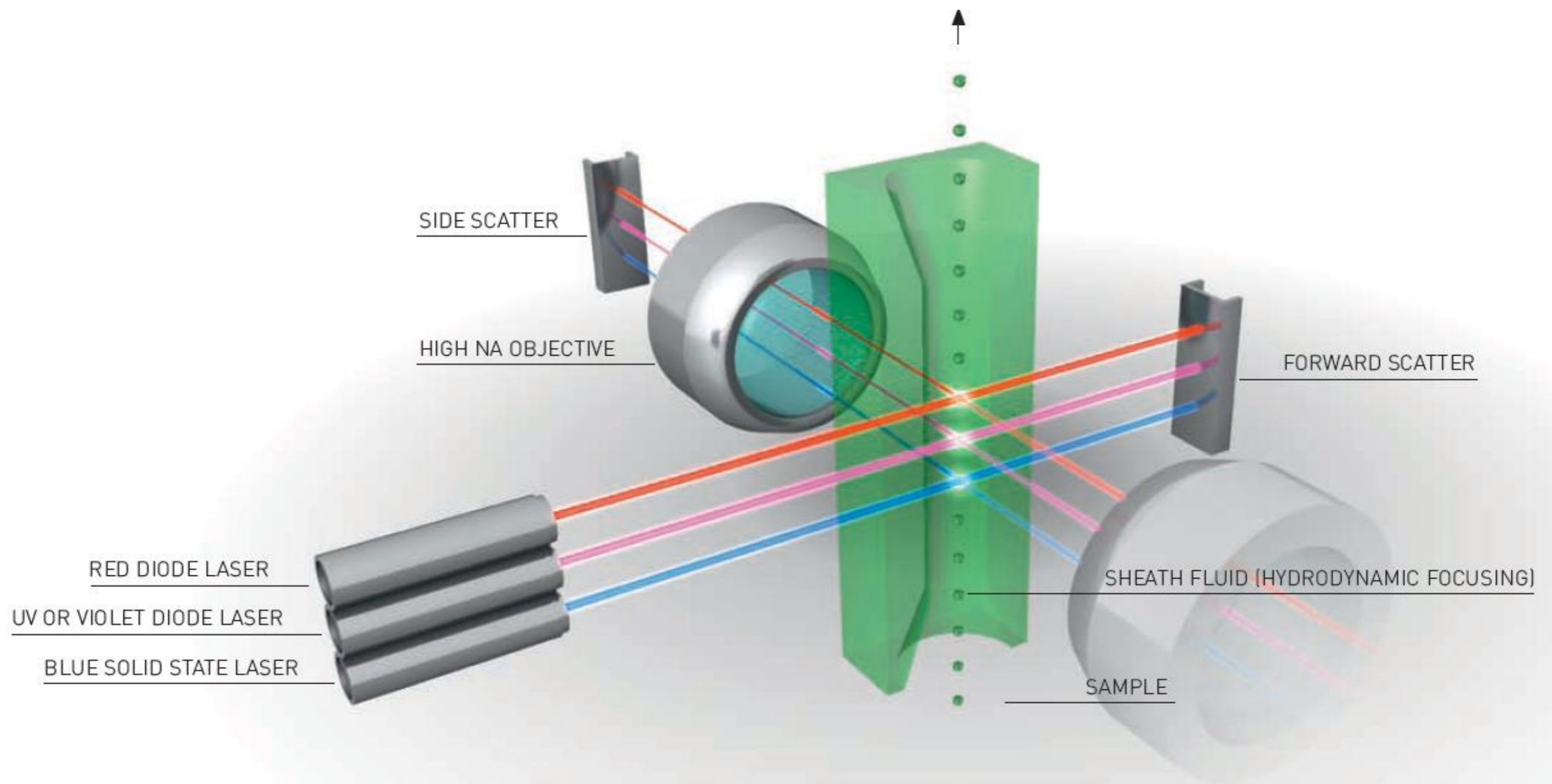
Now, let's flow:  
The Cube interior



# Flow cytometer felépítése



# Áramlási kamra



# Fluoreszcens jelek szeparálása

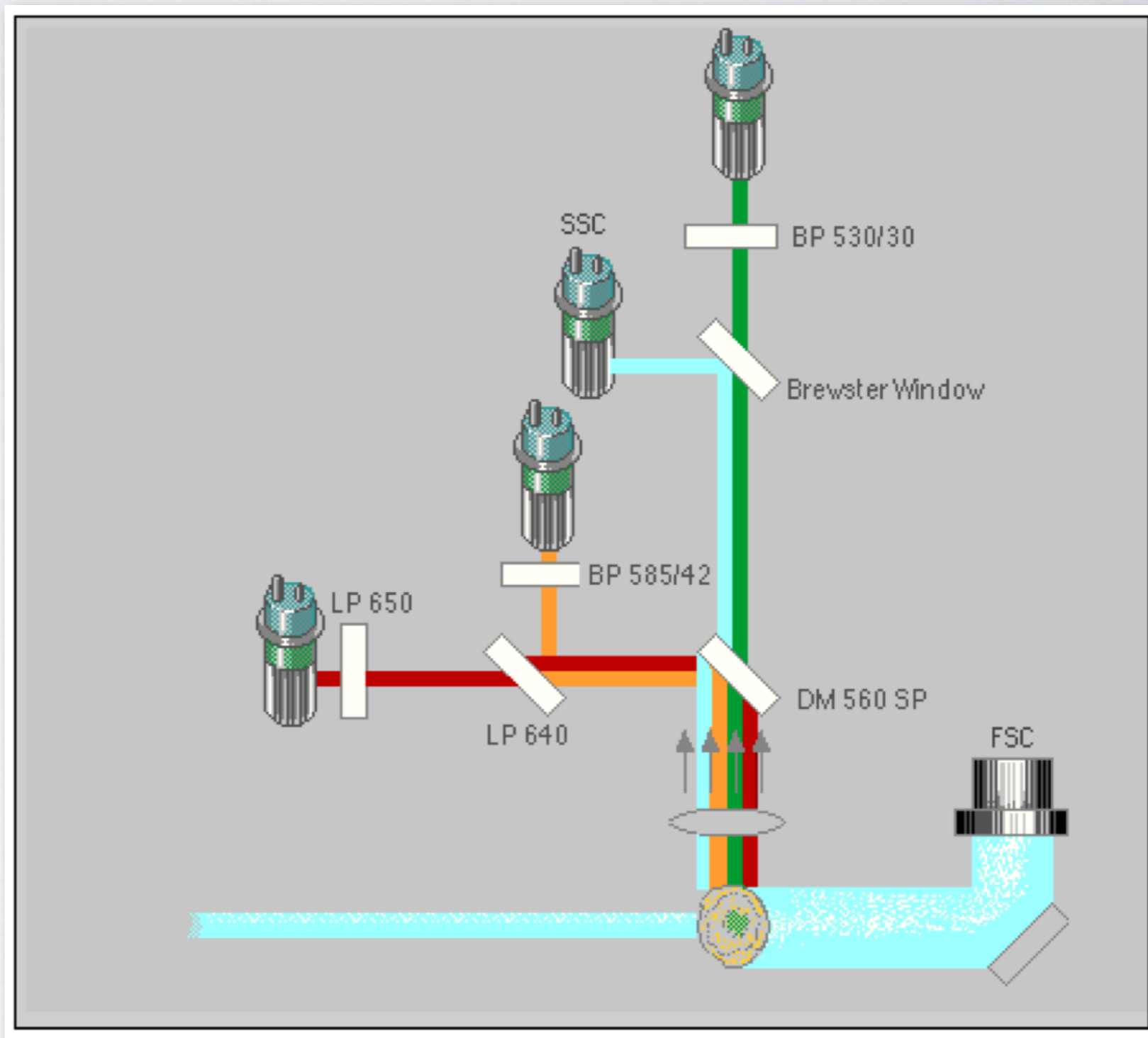
Band pass-sávszűrő: adott hullámhossz tartományban enged át. Pl. BP 530/30: 515-545 nm.

Dicroic mirror-dikroikus tükör: adott hullámhossznál szeparáló tükör

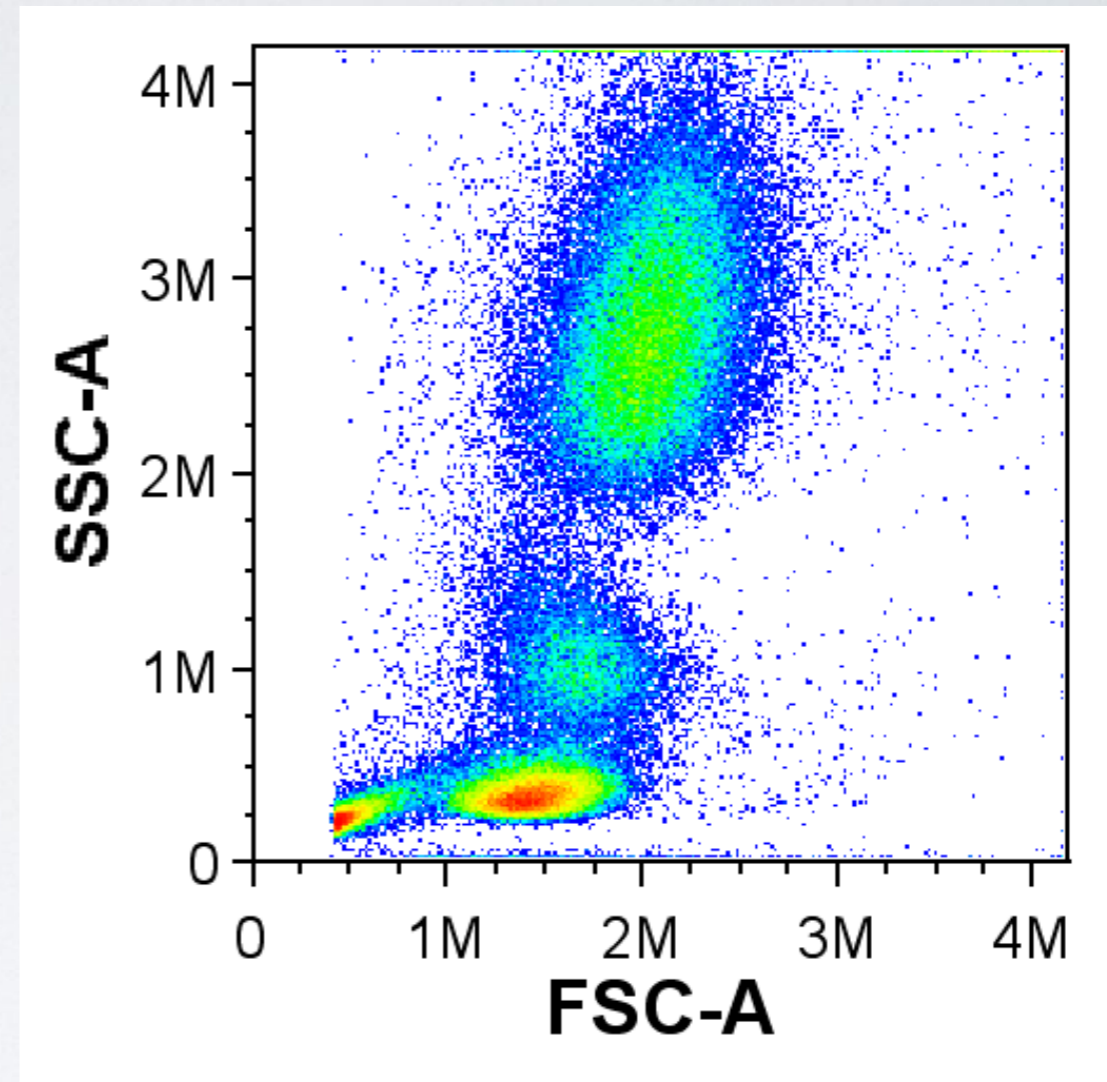
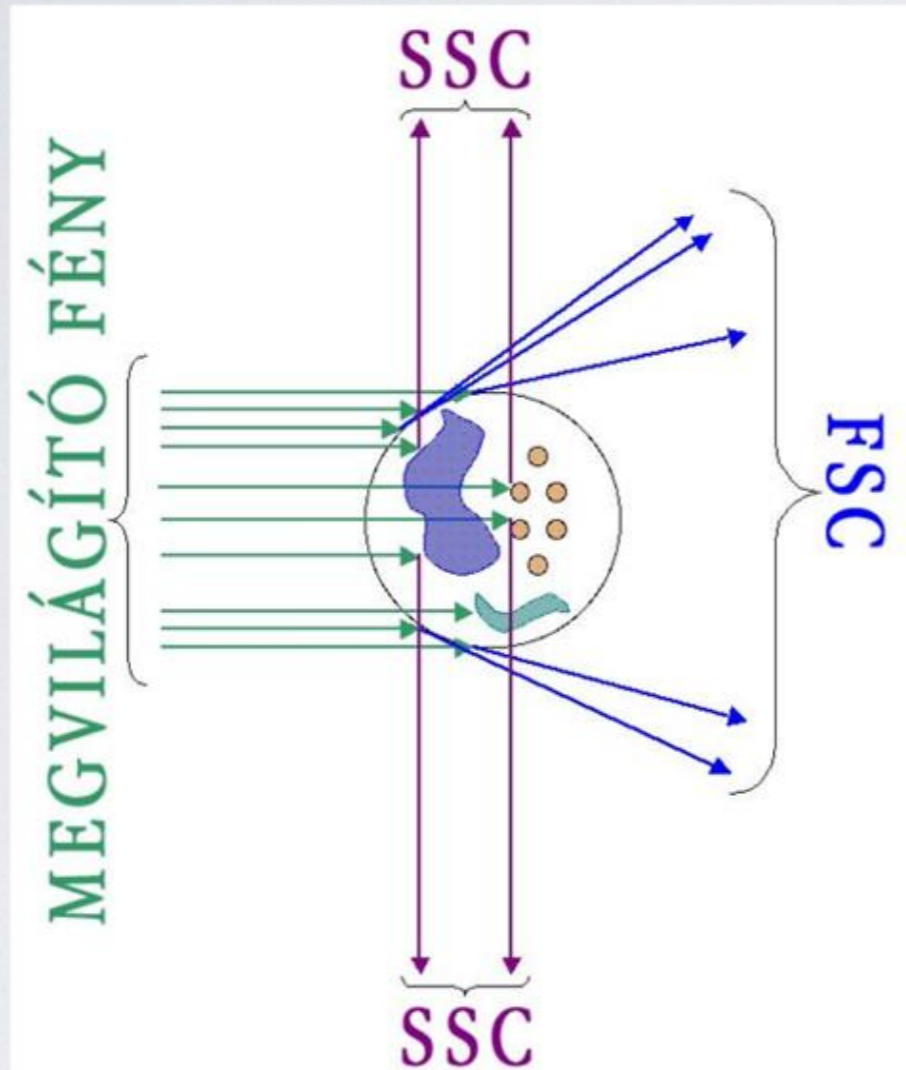
- SP short pass: adott hullámhossz alatt enged át, fölötte tükröz
  - LP long pass: adott hullámhossz fölött enged át, alatta tükröz
- Pl. DM 560 SP: 560 nm alatt enged át, fölötte tükröz

Long pass szűrő: adott hullámhossz fölötti mindent átenged, alatta elnyel. Pl. LP 650: 650 nm fölötti fényt átengedi, alatta elnyeli.

Brewster window: polarizált fényt (gerjesztő) tükrözi, a nem polarizáltat (fluoreszcencia) átengedi.



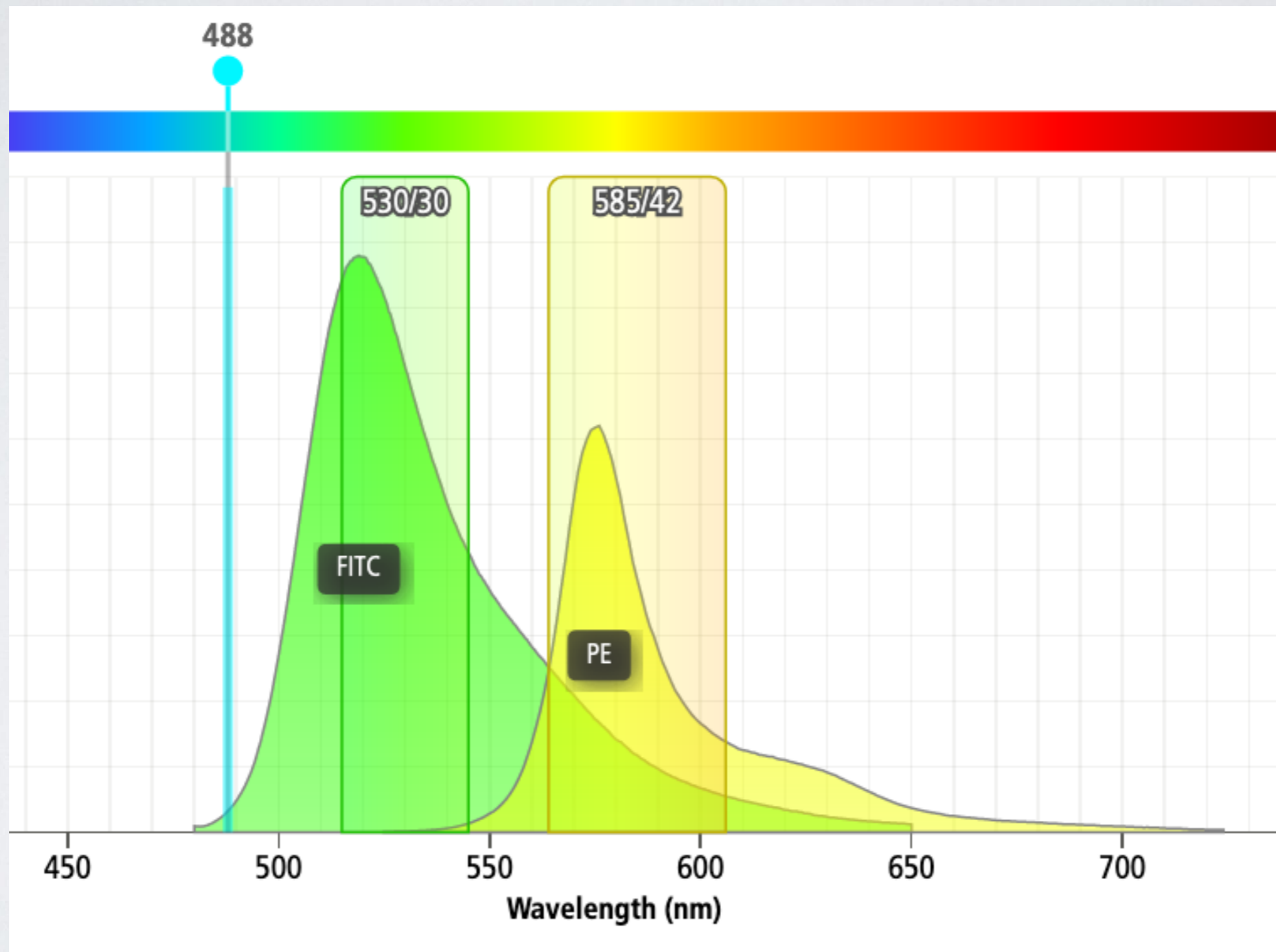
# Fényszórási jelek



**FSC** - forward scatter:  
előre irányuló fényszórás.  
Sejtmérettel korrelál.

**SSC** - side scatter:  
oldalra irányuló fényszórás.  
Granularitásra utal.

# Emissziós spektrum



<http://www.bdbiosciences.com/us/s/spectrumviewer>



# 17 színű FCM

## Seventeen-colour flow cytometry: unravelling the immune system

*Stephen P. Perfetto, Pratip K. Chattopadhyay and Mario Roederer*

The increasing need for polychromatic approaches to flow cytometry, coupled with rapid technological advances, has pushed the frontiers of flow cytometry beyond 12-colour systems. Recent breakthroughs have allowed the design and implementation of instruments that measure 19 parameters (17 fluorescent colours and 2 physical parameters). This article describes the instrumentation and considers the reagents, analysis and applications for this powerful, new extension of flow-cytometric technology.

Fluorescence-activated flow cytometry is one of the most powerful technologies that is routinely used in immunology. It allows not only for the rapid multiparametric analysis of cells on a cell-by-cell basis, but also for the viable separation (sorting) of highly purified populations of cells. Flow cytometry was born in the Herzenberg laboratory at

Stanford University (Stanford, United States) in the late 1960s<sup>1-3</sup>. Early cytometers measured three parameters (one fluorescence signal and two scattered-light signals); the power of multiparametric analysis to discriminate functionally distinct subsets of leukocytes drove the technology to the commonly used four-colour machines that can distinguish six parameters for each cell. This four-colour analysis was first performed in the mid-1980s. In the 1990s, further technological advances were made necessary by the growing appreciation of researchers for the complexity of the immune system; a systematic series of developments of hardware, software and chemistry were spearheaded by the Herzenberg laboratory to create machines capable of measuring more than ten colours<sup>4</sup>.

This technology has since revealed that there are hundreds of phenotypically distinct cell types in the peripheral blood of humans<sup>5</sup>.

parameters (forward and side scatter) and two fluorescence parameters are sufficient. Forward- and side-scatter characteristics can be used to distinguish monocytes and granulocytes (high side scatter and high forward scatter) from platelets and erythrocytes (low side scatter and low forward scatter). Lymphocytes and circulating dendritic cells show intermediate forward and side scatter and can be further segregated by lineage (that is, B cells, T cells or natural killer (NK) cells) using one or more fluorescence parameters. For example, T cells can be identified using fluorescently labelled antibodies specific for the CD3 cell-surface marker and either the CD4 or CD8 cell-surface markers (two-colour flow cytometry).

Within T-cell populations, various markers have been proposed to distinguish naive, effector- and memory-cell populations. These markers are often paired (for example, CD45RA and CD62 ligand (CD62L), co-expression of which identifies naive T cells) to create the simple phenotyping schemes commonly used in four-colour experiments. However, attempts to describe the complex T-cell compartment using these phenotyping schemes have raised many questions. For example, most of the common markers that are used to identify the stages of T-cell

# 40+ színű FCM

## Toward 40+ Parameter Flow Cytometry, CYTO 2014

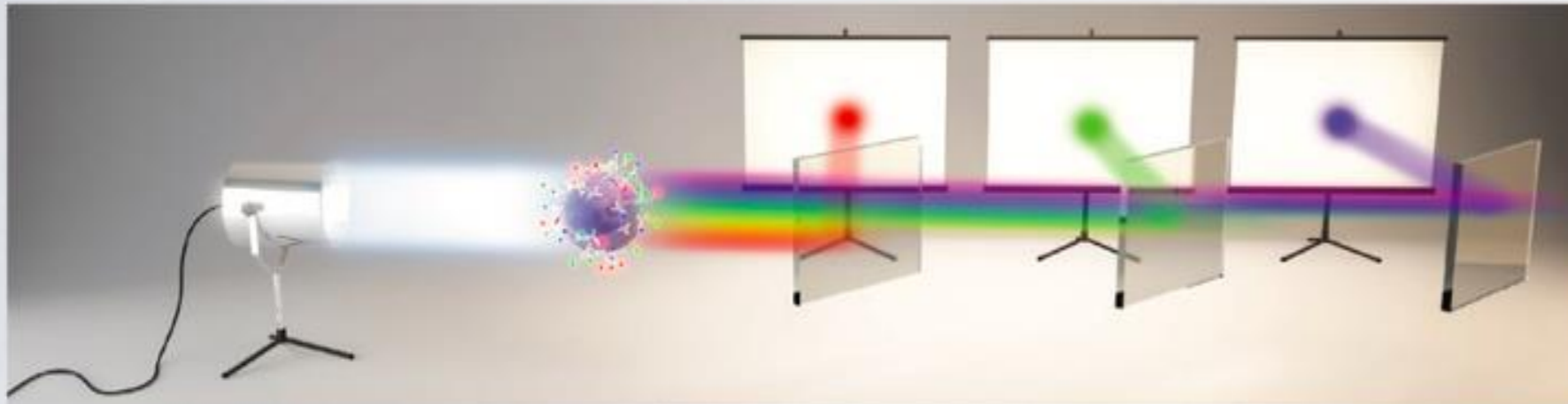
**Pratip Chattopadhyay<sup>1</sup>, Stephen Perfetto<sup>1</sup>, Brent Gaylord<sup>2</sup>, Alan Stall<sup>2</sup>, Larry Duckett<sup>2</sup>, Julie Hill<sup>1</sup>, Richard Nguyen<sup>1</sup>, David Ambrozak<sup>1</sup>, Robert Balderas<sup>2</sup>, Mario Roederer<sup>1</sup>**

<sup>1</sup>ImmunoTechnology Section, Vaccine Research Center, National Institutes of Health, Bethesda, MD, United States, <sup>2</sup>BD Biosciences, San Diego, CA, United States

- This system uses five tunable lasers: 355nm/60mW, 405nm/1000mW, 488nm/500mW, 532nm/1000mW and a 628nm/1000mW.
- laser power titration curves, we can track changes in staining index and quantitatively identify the optimal laser power for a wide variety of fluorochromes
- we report our testing of 43 fluorochromes (commercially available or in advanced stages of development), and rank them by staining index

# Spektrális flow cytometer

## A Conventional filter-based Flow Cytometry



A hagyományos flow cytometerek az emissziós tartomány egy részét detektálják.

## B Spectral-Analysis Technology

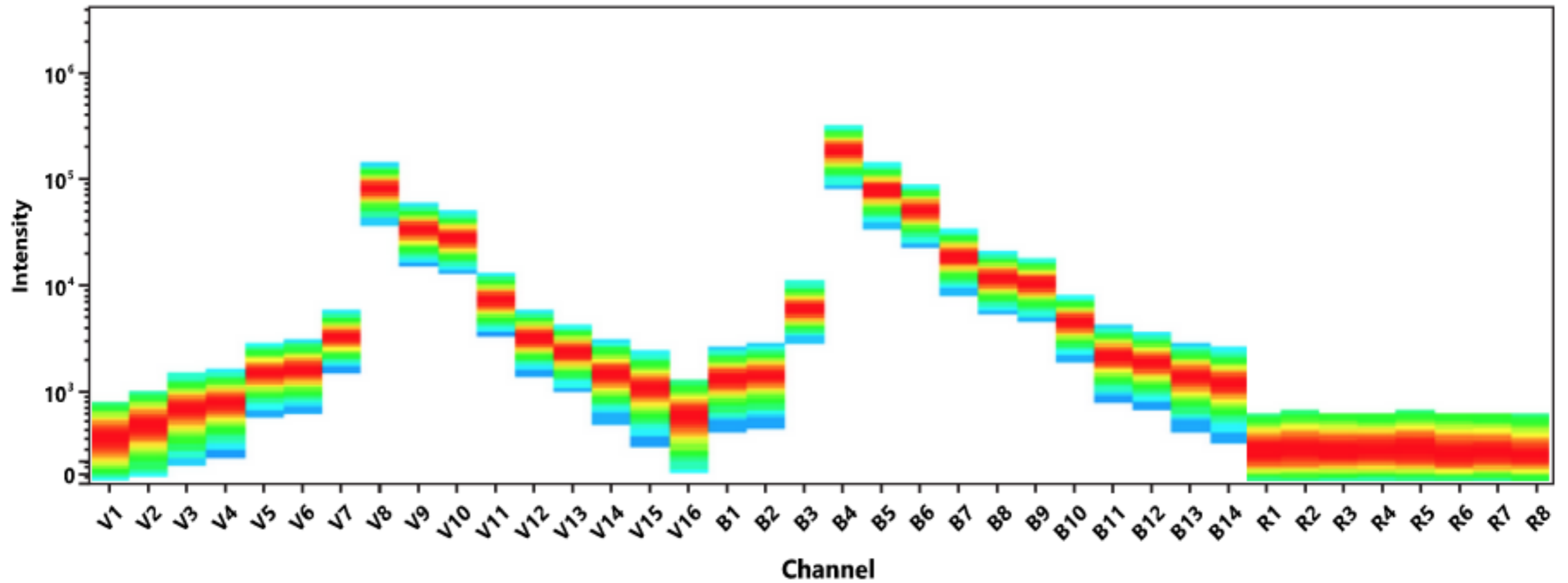


A spektrális flow cytometerek a teljes emissziós tartományban detektálnak.

**Figure 1.** Concept of Spectral-Analysis in Flow Cytometry

# Spektrális mintázat

PE  $\approx$



# 10 vs. 20 színű AML panel

backbone  
markers

B5+R3+V2=10c	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8	FL9	FL10
AML1 neutrofil (APL)	15	117	11b	45	33	13	64	34	Syto41	HLA-DR
AML2 monocita	4	117	14	45	33	13	64	34	Syto41	HLA-DR
AML3 aberráns markerek, LSC	56	117	19	45	33	7	38	34	Syto41	HLA-DR
AML4 eritroid, megakario, pDC, baso	MPO	117	71	45	33	123	61	34	Syto41	HLA-DR

V8+B6+R5 = 20c	BV421	Syto41	BV510	BV570	BV605	SB645	BV711	BV785	FITC	PE	PD594	PerCP	PerCP-Cy5.5	PE-Cy7, PE-Vio770	APC	Spark NIR 685	AF700	PE-Fire744	APC-Vio770	APC-Fire810
AML1	CD13	S41	CD14	CD19	CD56	CD61	15	HLA-DR	MPO	CD117	CD11b	38	CD71	CD33	CD123	CD7	CD64	CD4	34	CD45

# Immunfenotipizálás

## **Definíció:**

Adott minta sejtjeinek immunmarkerekkel való vizsgálatát és jellemzését jelenti.

## **Alkalmazása:**

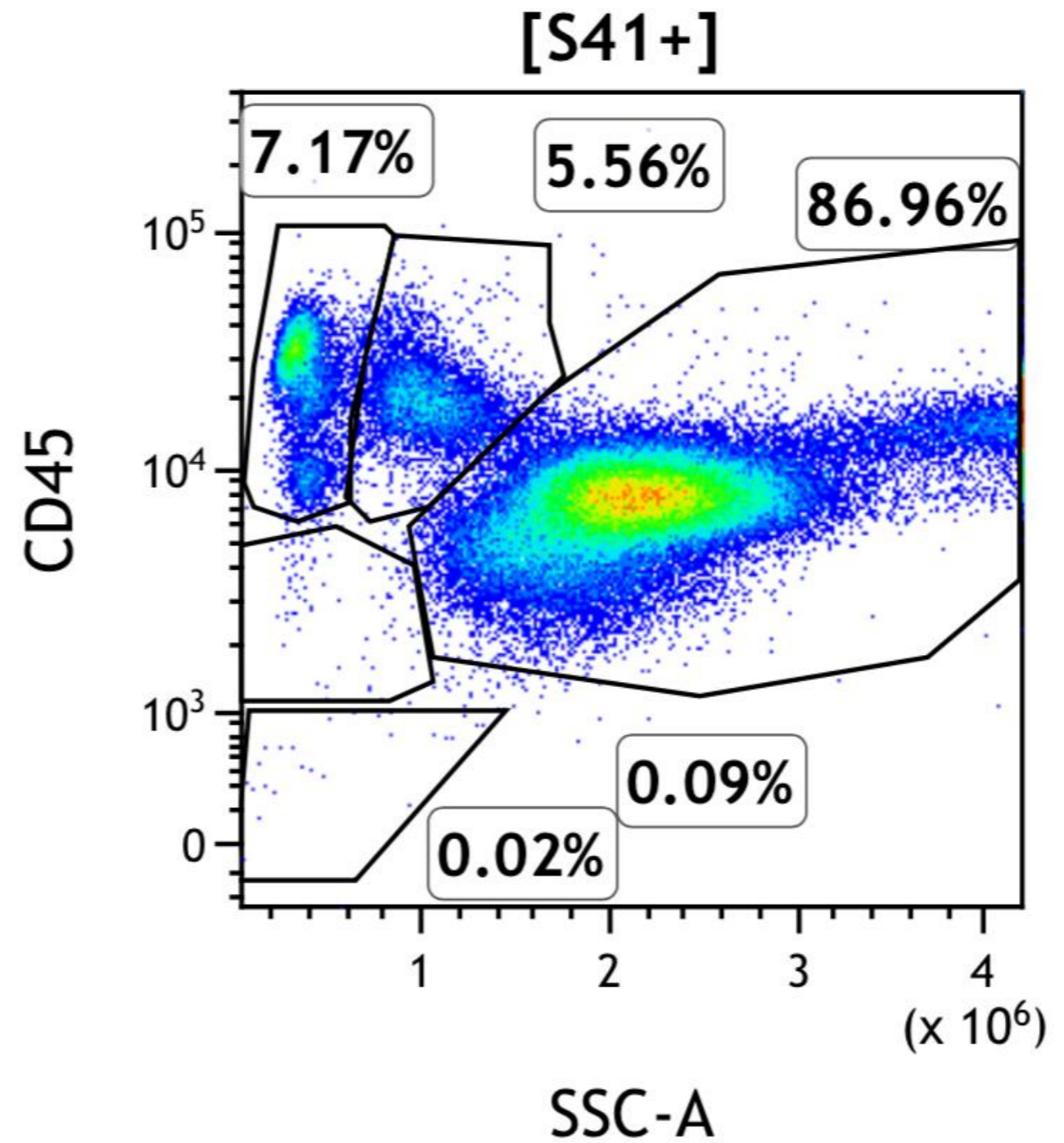
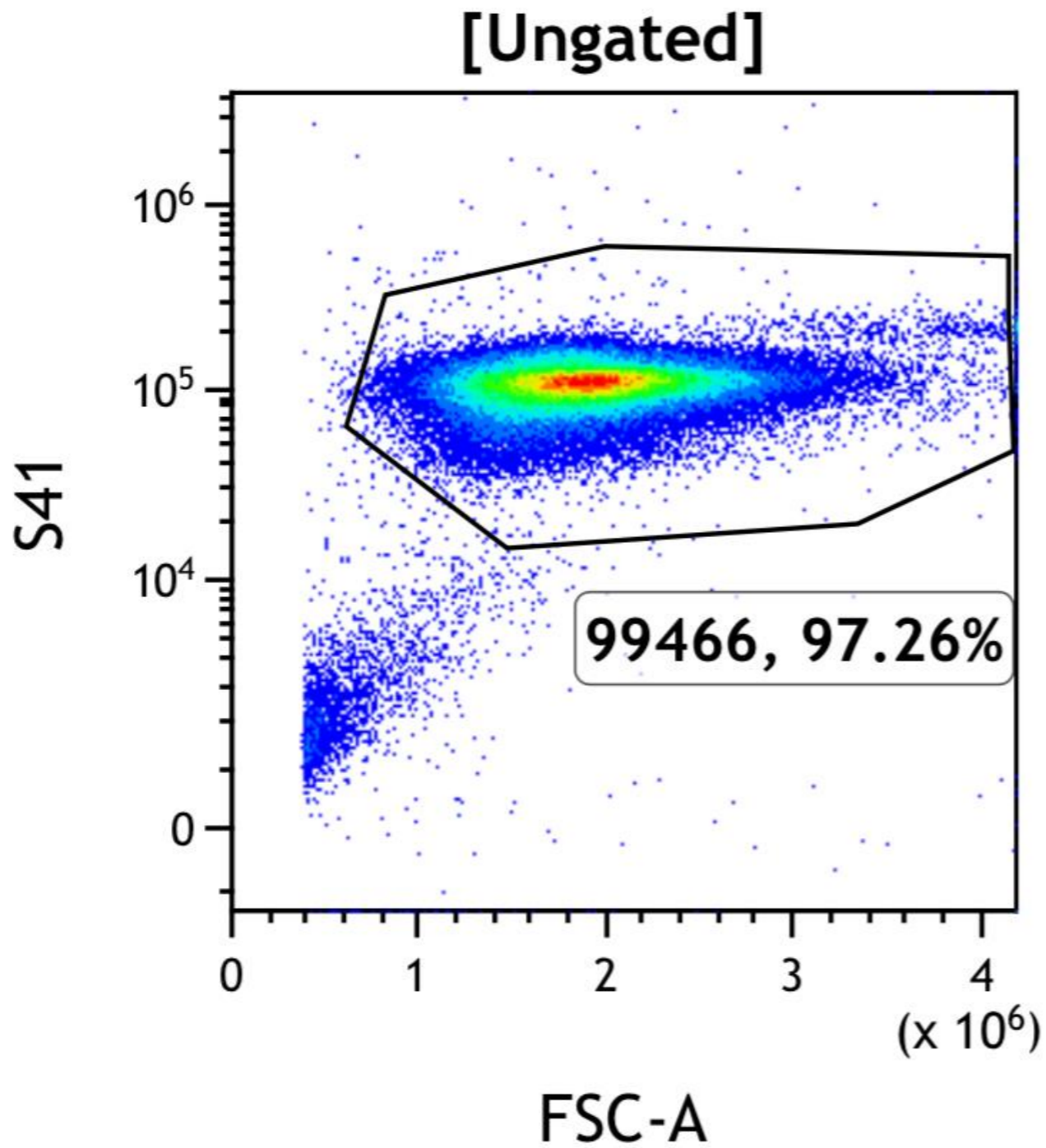
Sejtfelszíni és intracelluláris antigének kimutatásával, jellemző antigén expressziójuk alapján a különböző sejtípusok,

- azonosíthatók,
- egymástól elkülöníthetők,
- kvantitálhatók.

# Immunfluoreszcens jelölés



# Kapuzás - gating



Sejtpopulációk szoftveres elkülönítése mért paraméterek alapján.



# Immunfenotipizálás onkohaematológiai alkalmazásai

1. Az **alapdiagnózis felállításában** segít betegségre jellemző sejtpopulációk azonosítása révén.
2. **Betegség monitorozása:** abnormális populációk mennyiségi változásának vizsgálata kezelés során. Minimális reziduális betegség meghatározása.
3. **CD34+ őssejt koncentráció meghatározás** autológ őssejt transzplantációhoz.

# FCM diagnosztikai stratégia

Új beteg

Sürgős  
kenet



**Adekvát** klinikai  
**adatok** és irány  
**diagnózis**

**Kevés** klinikai **adat**  
és/vagy **bizonytalan**  
irány **diagnózis**

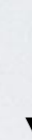
Célzott  
panel  
alkalmazása

Tájékozódó  
panel  
alkalmazása

Célzott panel  
alkalmazása

Ismert beteg

Sürgős  
kenet



Célzott, szűkített,  
MRD panel  
alkalmazása

# Fenotipizáláshoz használt immunmarkerek

## 1. Sejtvonal markerek

- B-sejt: CD19, CD20, CD22, CD23, CD24, CD38, CD138
- T-sejt: CD2, CD3, CD4, CD5, CD7, CD8
- NK-sejt: CD16, CD56, CD57
- Myeloid, monocytoid: CD13, CD14, CD15, CD16, CD33, CD64, MPO

## 2. Érés markerek

- CD45, CD10, CD34, CD117, TdT

# Fenotipizáláshoz használt immunmarkerek

## **3. Klonalitás markerek:**

- B-sejt: immunglobulin könnyűláncok - kappa, lambda
- T-sejt: TRBC-1, TRBC-2

## **4. Egyéb (abnormális expressziójú, prognosztikai stb.) markerek:**

- CD11c, CD25, CD103, CD123, CRLF-2, CD304, CD38,  
CD200, CD7, CD56

# Antitestek panelek

## 1. B-sejtes neopláziák

- **CLL/MCL:** CD5, CD19, CD20, CD23, CD38, CD200, kappa, lambda, CD45
- **HCL:** CLL + CD11c, CD25, CD103
- **MZL:** CLL + CD11c
- **FCL:** CLL + CD10
- **MM:** CD38, CD138, CD56, CD19, CD117, CD20, CD45

# Antitestek panelek

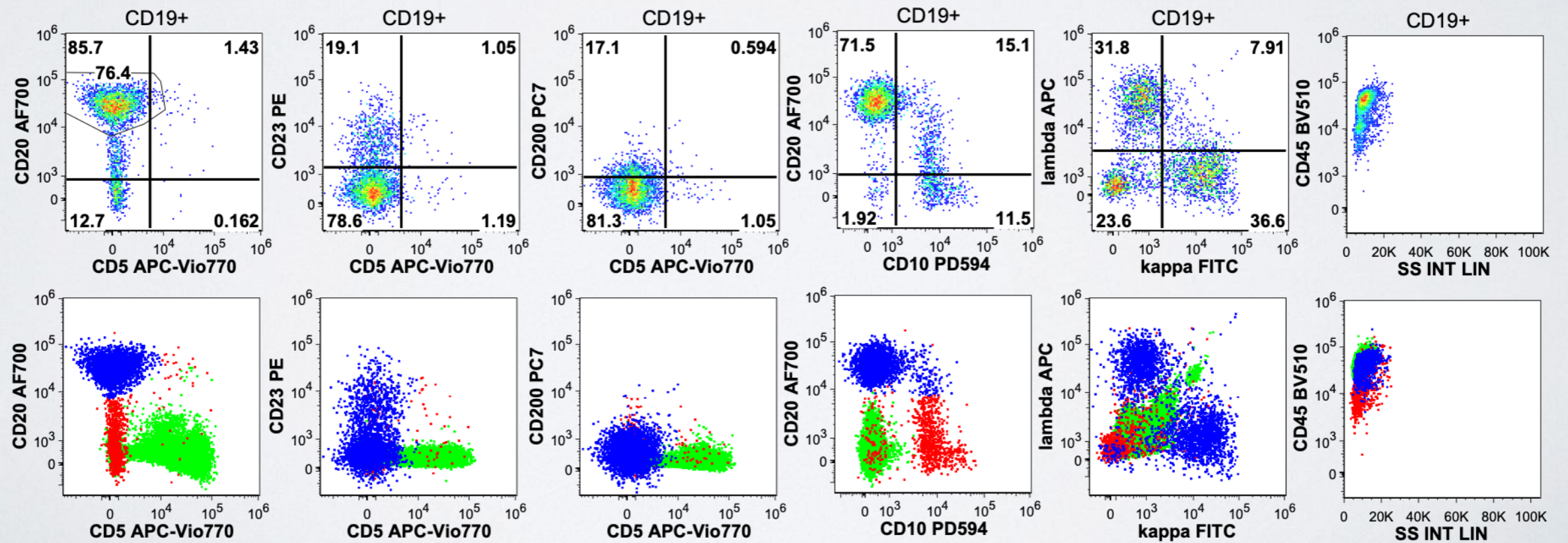
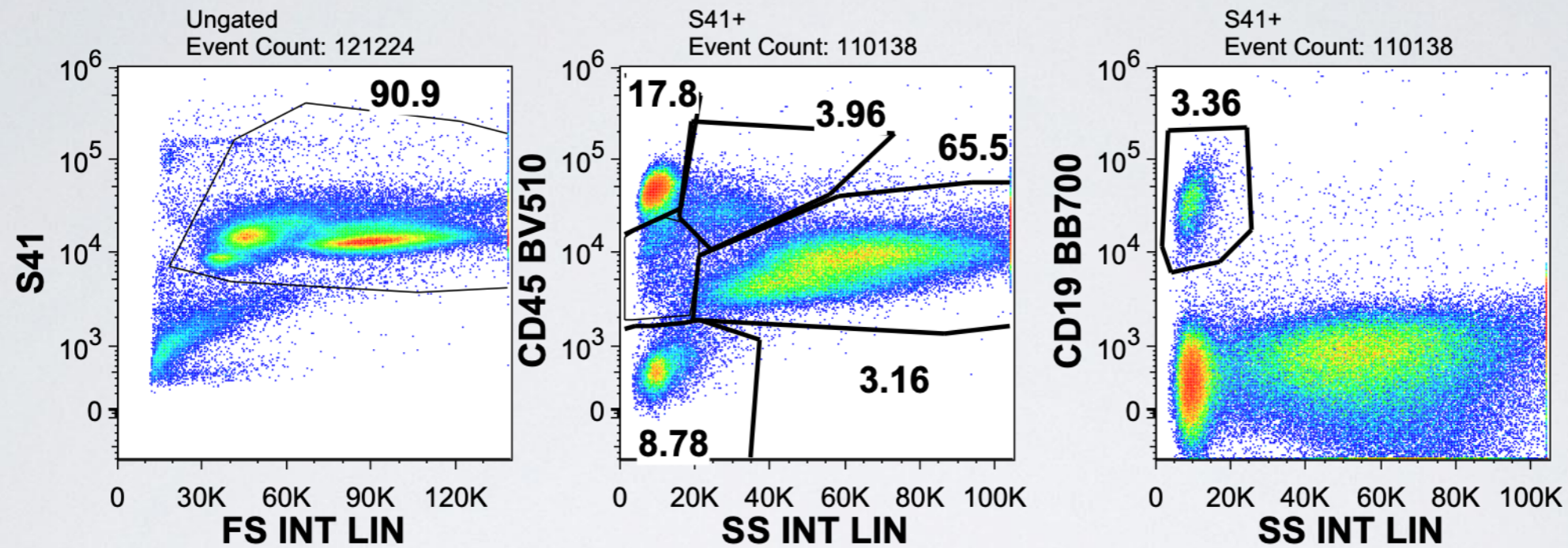
## 2. ALL

CD45, CD10, CD34, TdT, CD19, CD20, cy-IgM, s-IgM, kappa, lambda, CD2, CD3, CD4, CD5, CD7, CD8, CD1a, CD13, CD15, CD33, CD64, CD58, CD66c, CD123, CRLF-2, CD304

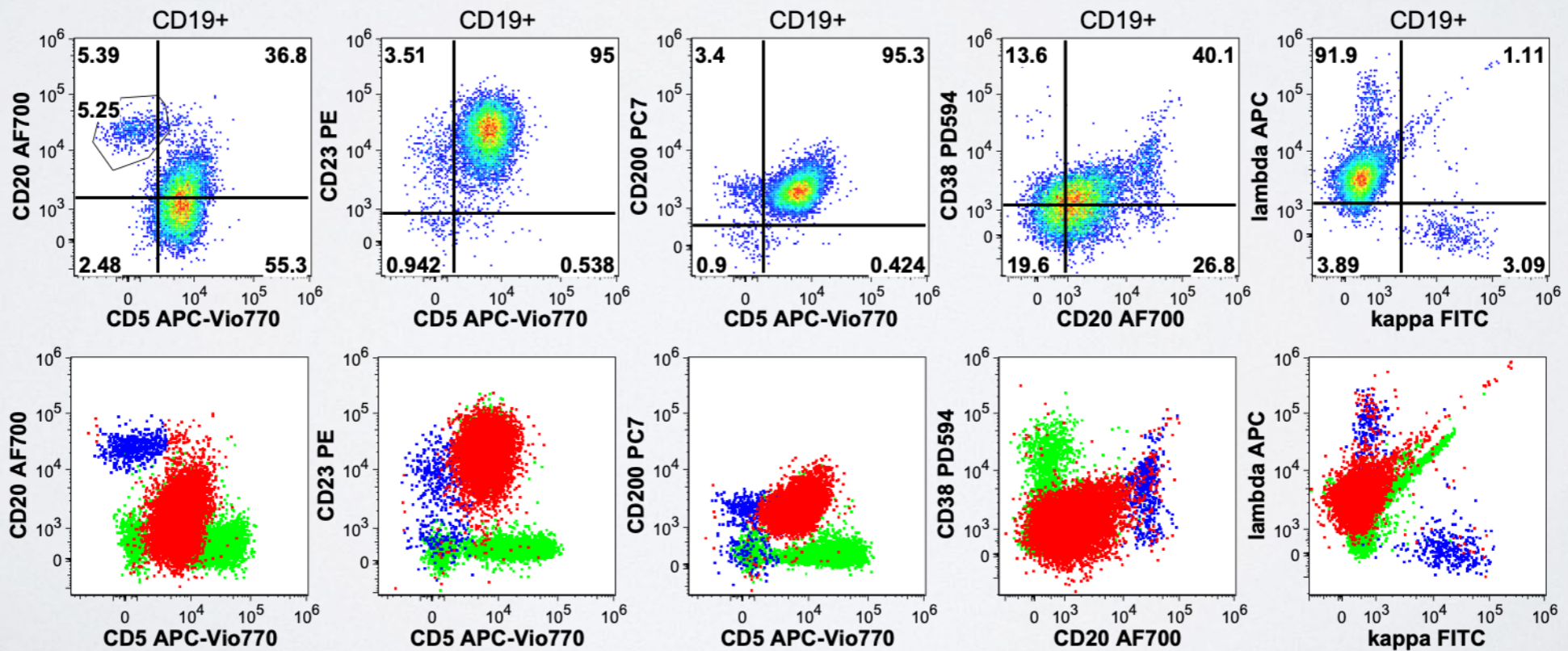
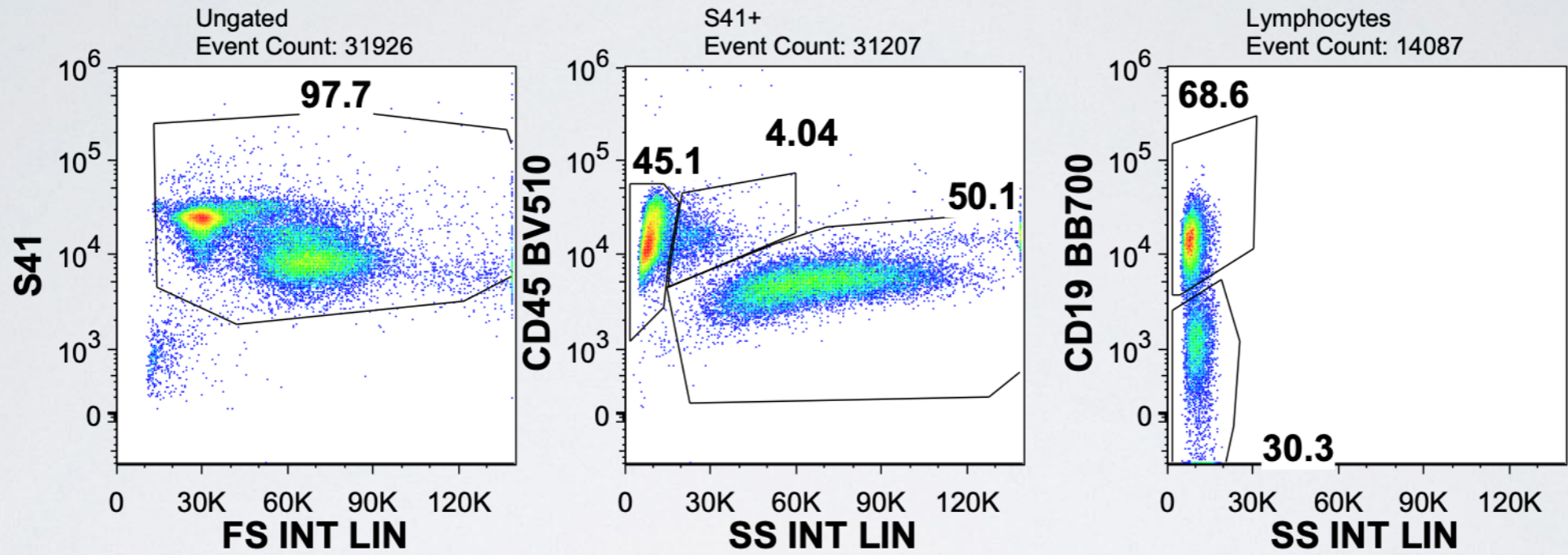
## 3. AML

CD45, CD10, CD34, CD19, CD2, CD3, CD7, CD13, CD14, CD64, CD33, CD11b, CD15, CD61, HLA-DR, CD56, CD117, MPO, CD123

# B-sejt érés normál csontvelőben

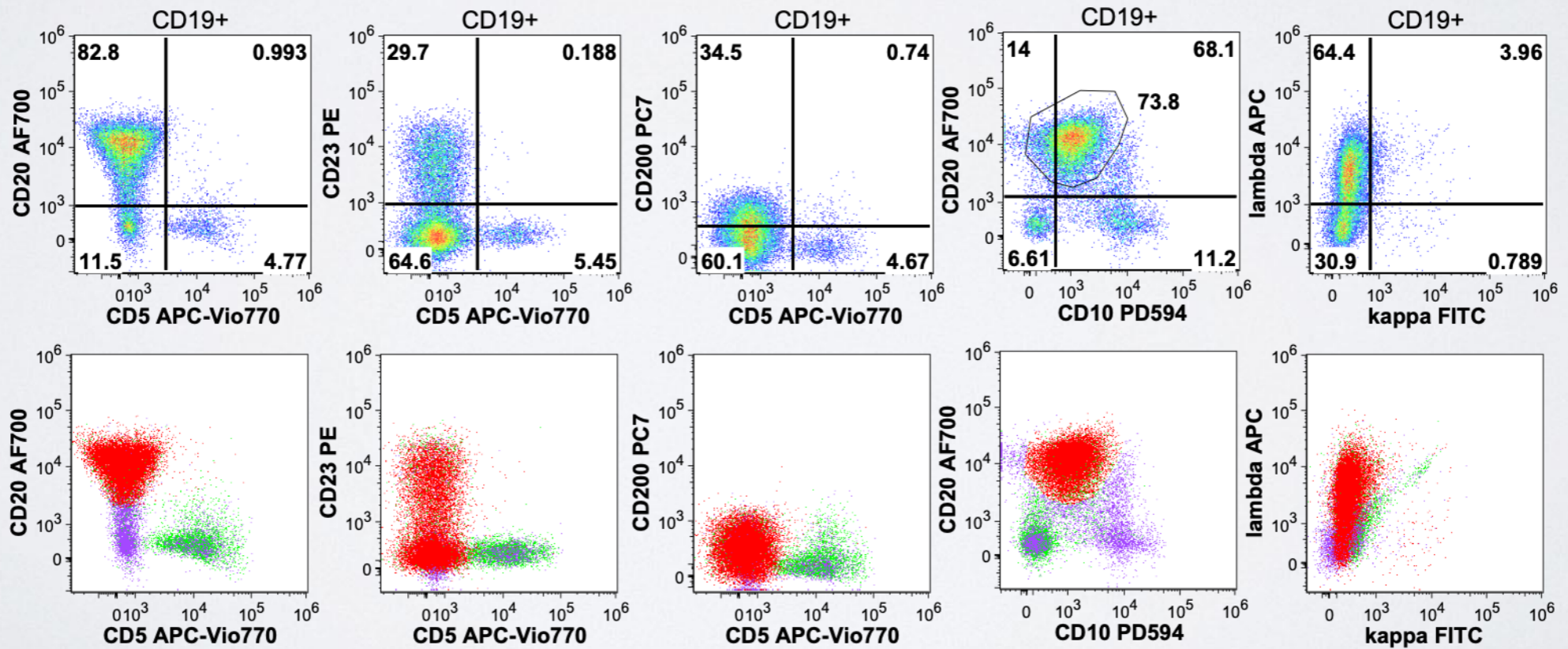
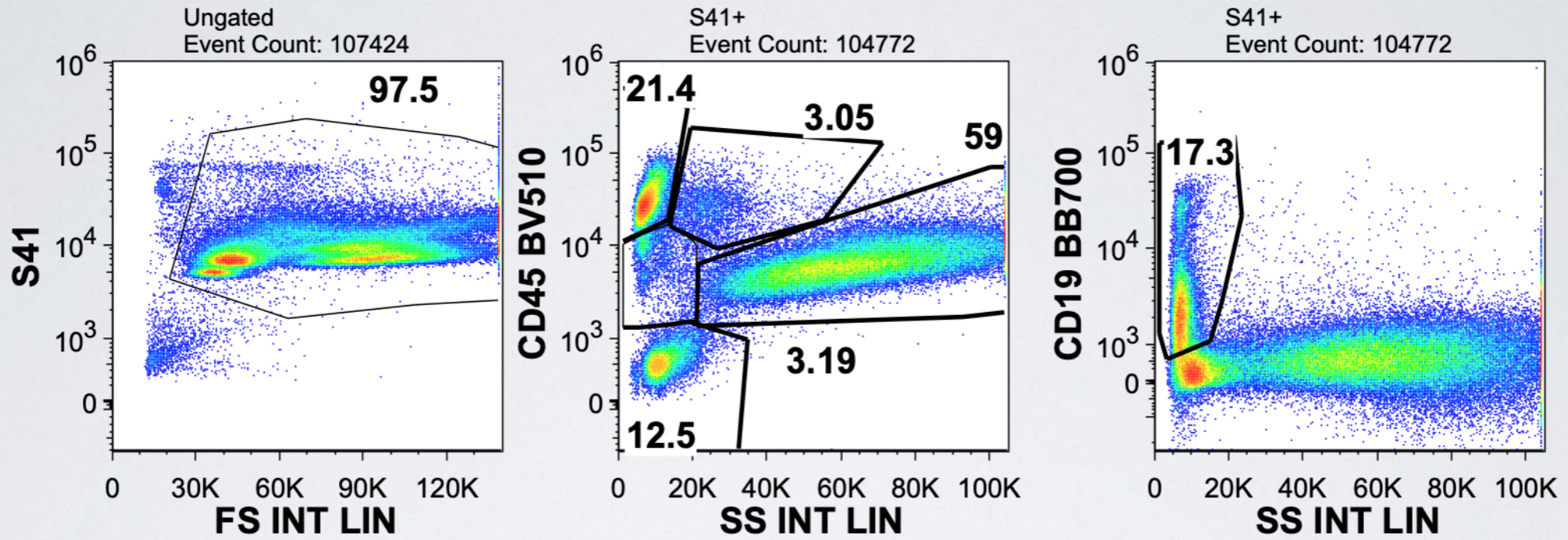


# CLL, vér

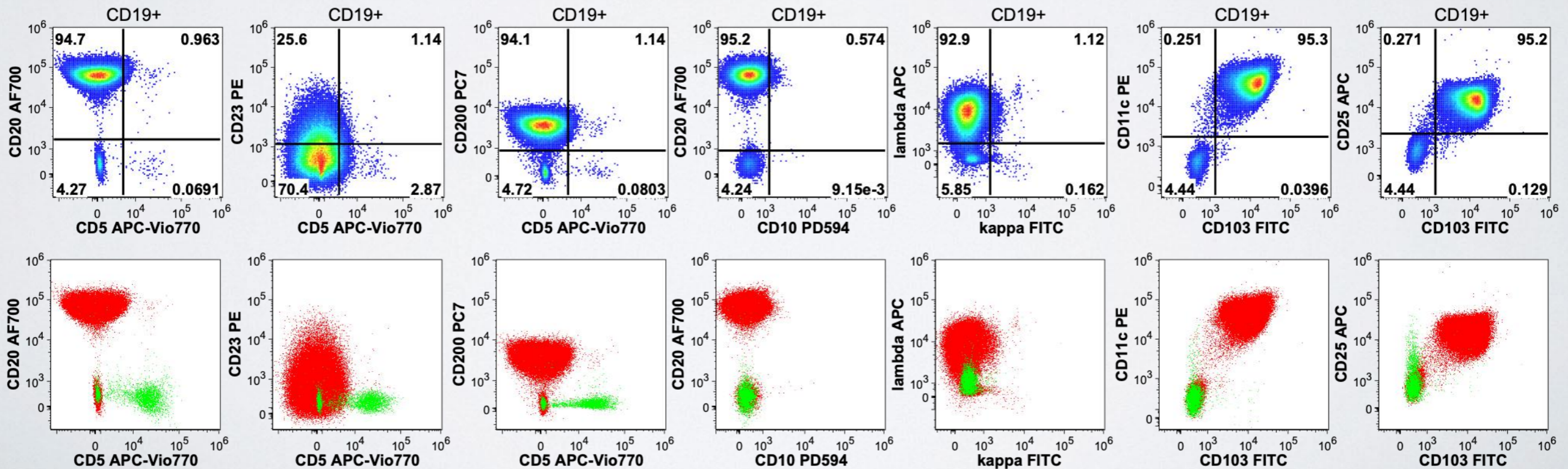
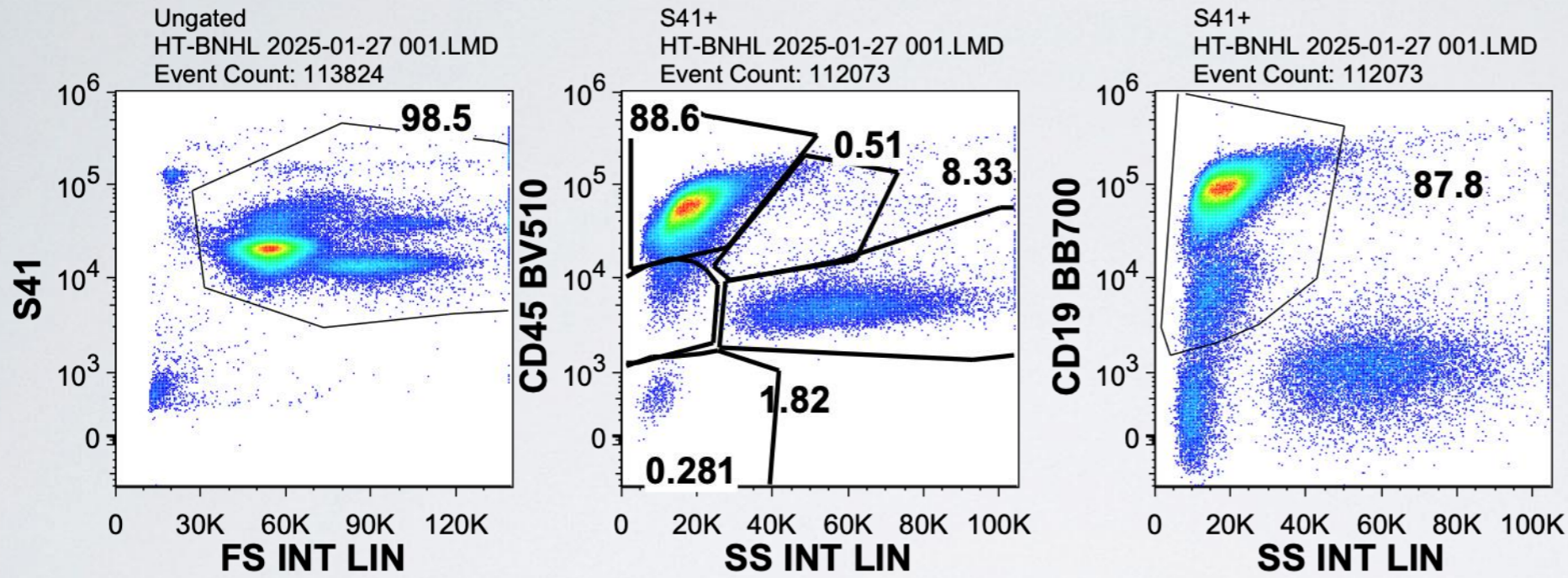




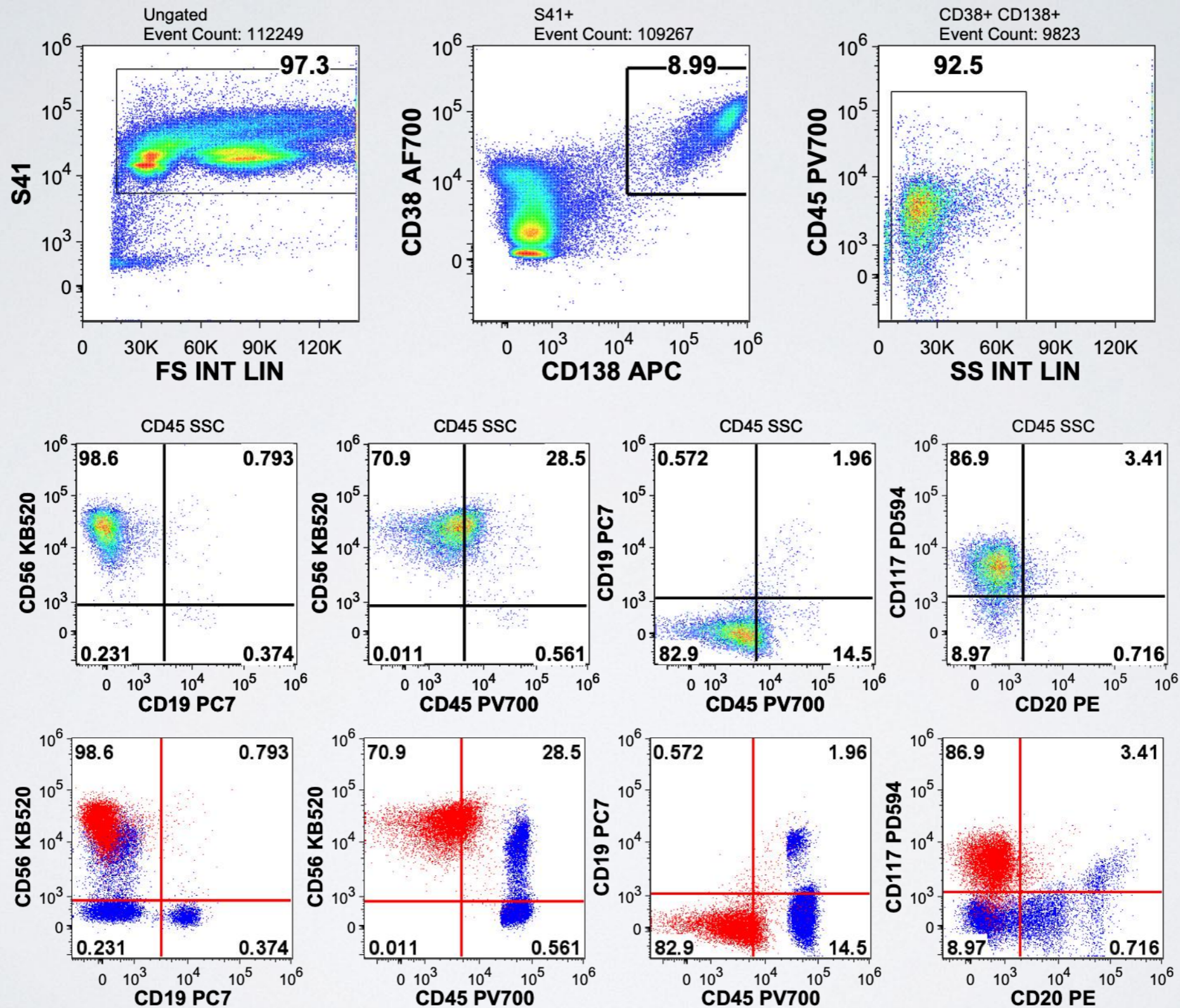
# FCL



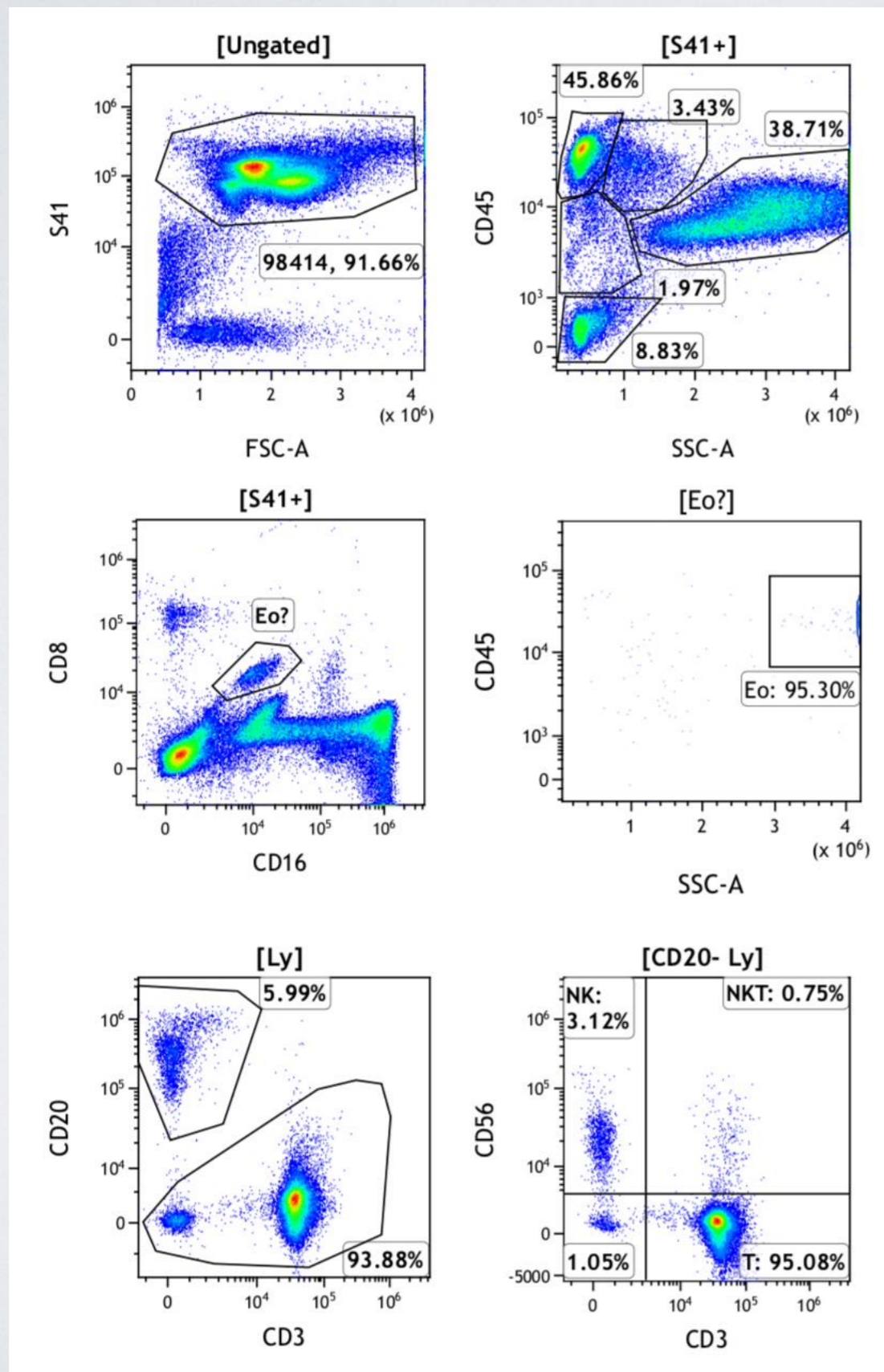
# HCL, vér



# Plazmasejtes myeloma



# T-sejtes lymphoproliferációk

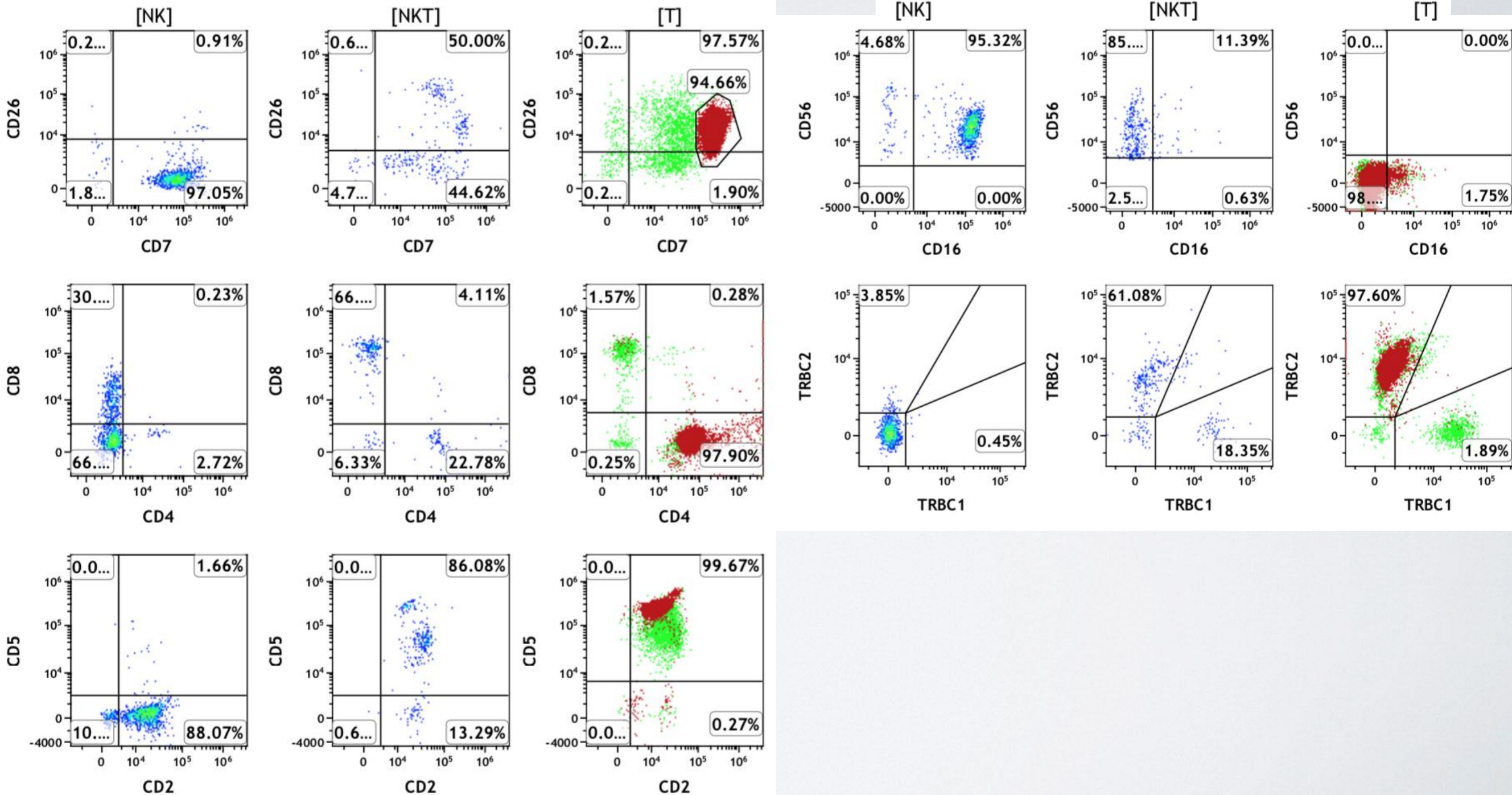


## Sejt arányok

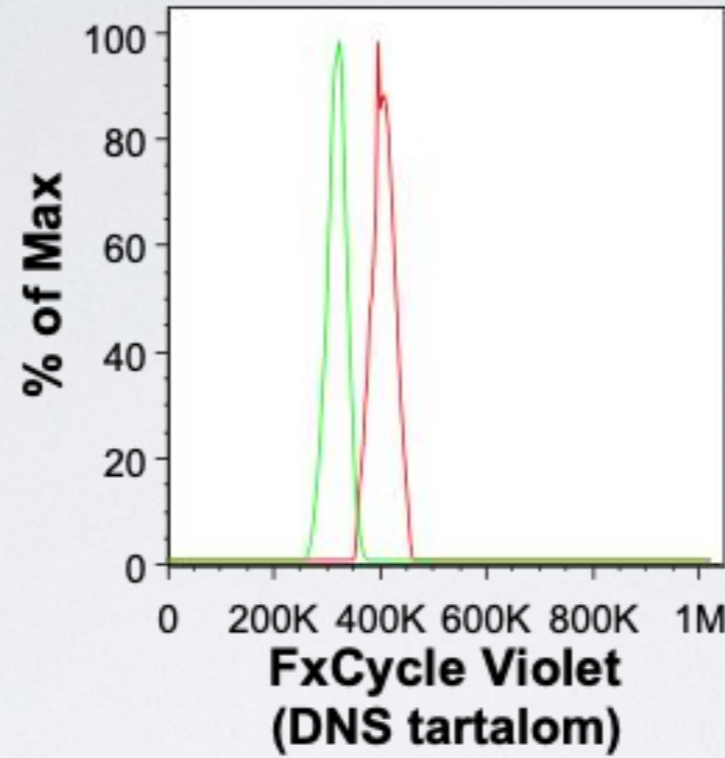
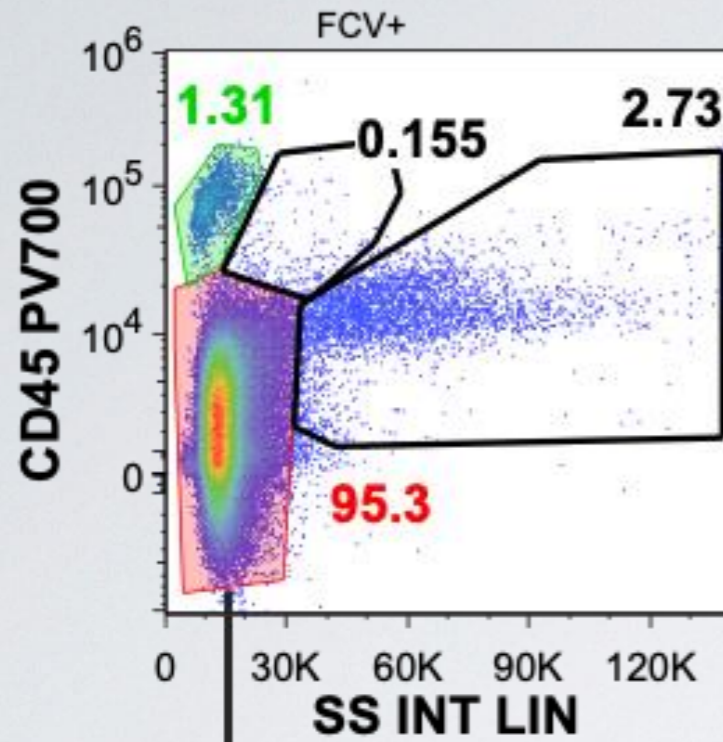
Ly%	45.86
Mo%	3.43
My%	38.71
Eo %	1.57
Blast%	1.97
CD20+ B	2.75
NK%	1.35
NKT%	0.32
T%	40.93
T: CD4/CD8	62.50
Ly+Mo+My+Blast+Er%	98.79

# T-sejtes lymphoproliferációk

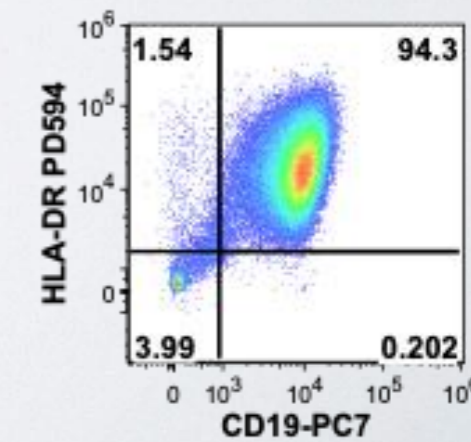
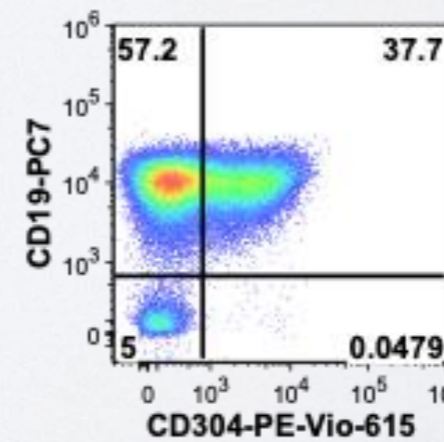
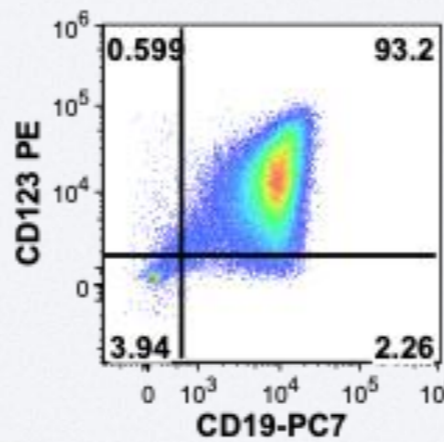
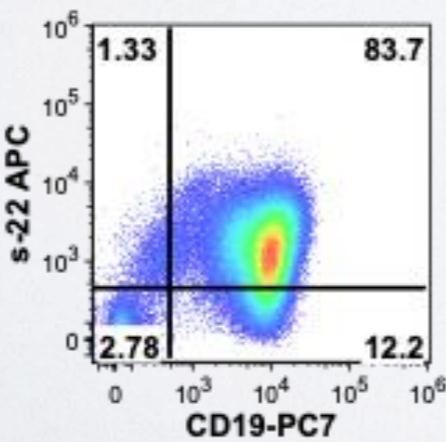
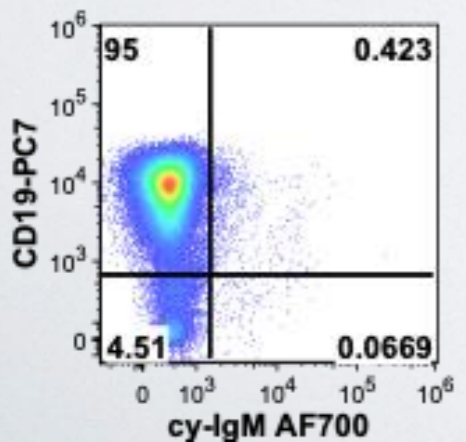
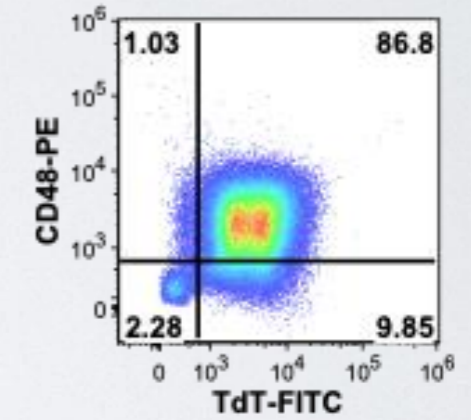
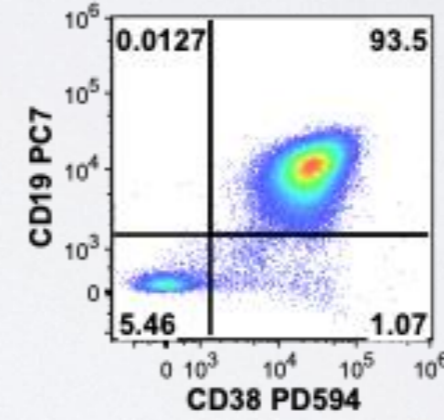
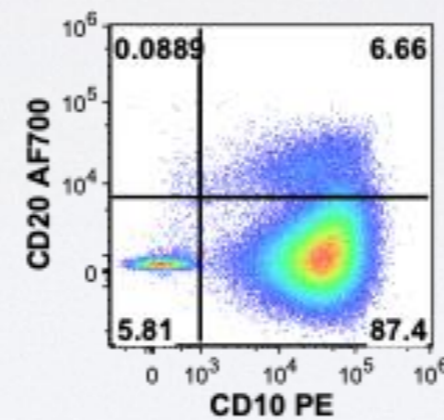
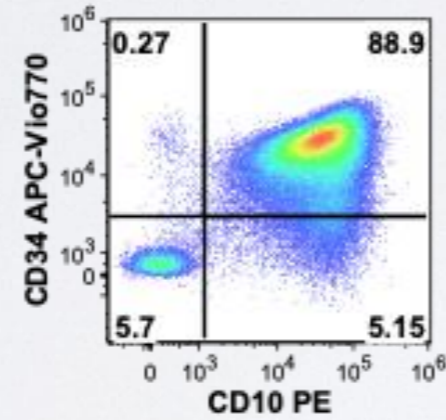
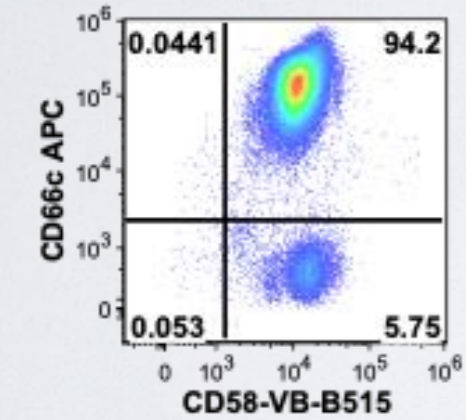
## Perifériás T-sejtes lymphoma



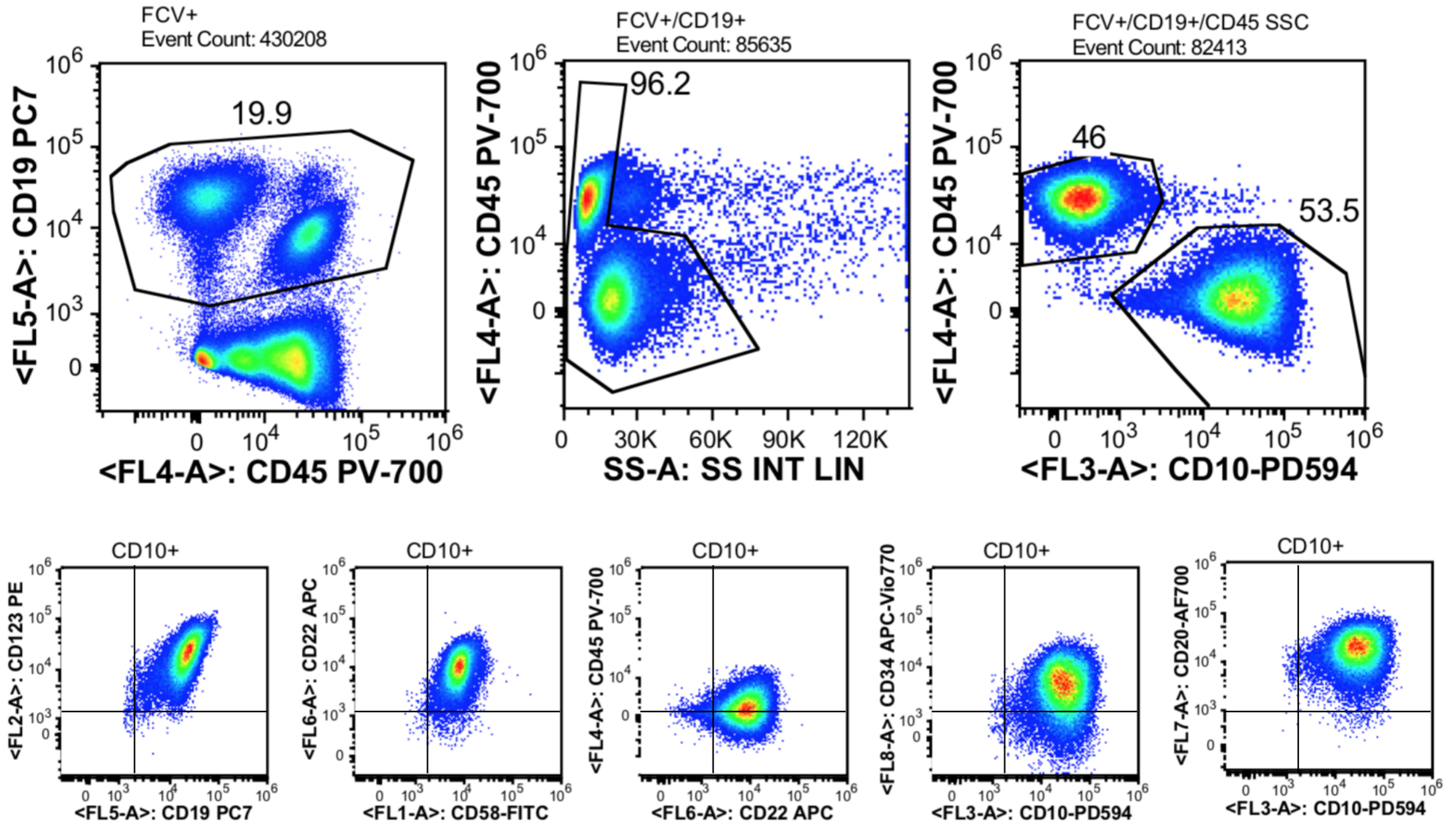
# B-ALL, csontvelő



DNS index: 1,26

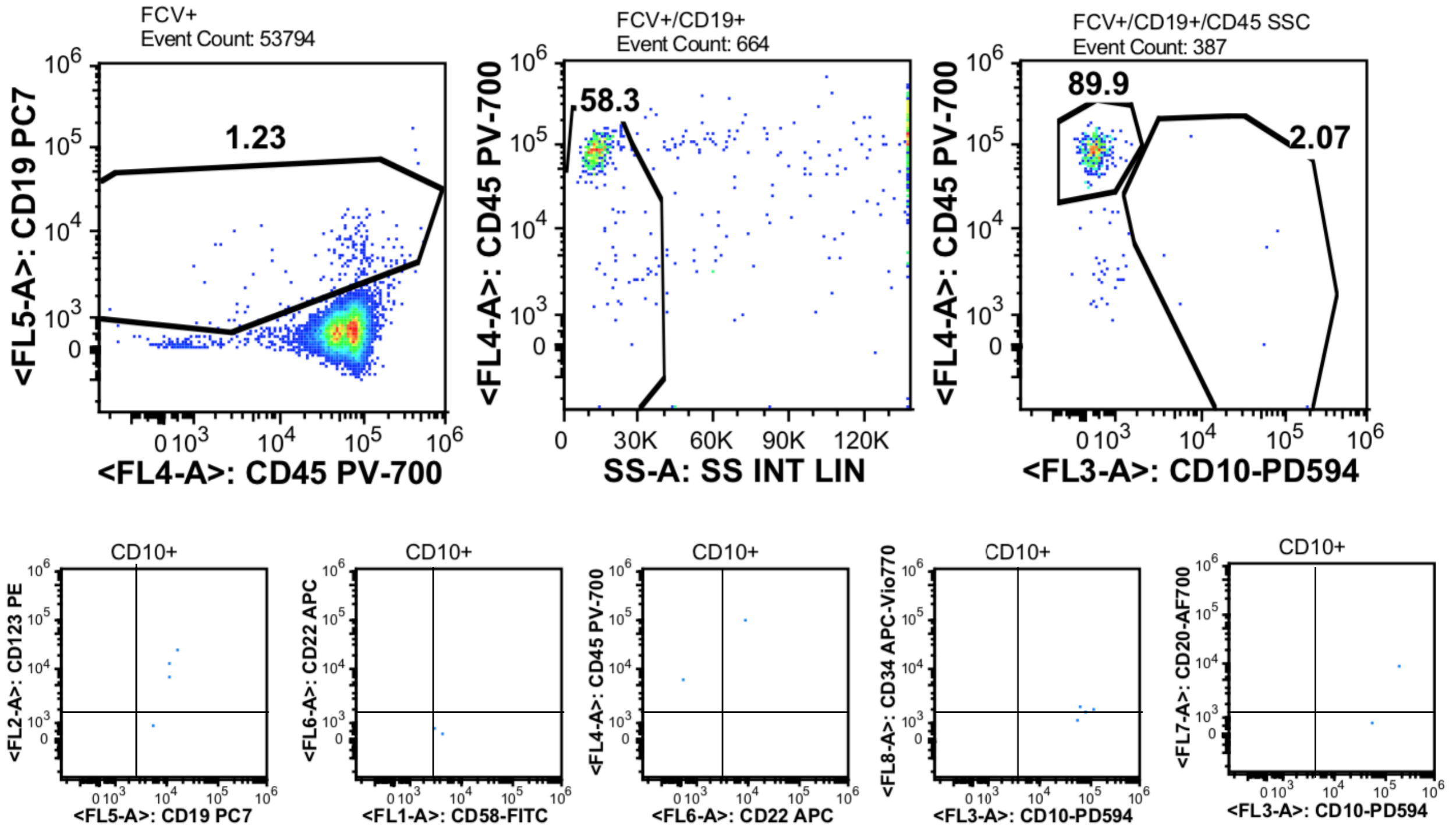


# B-ALL MRD, 15. nap



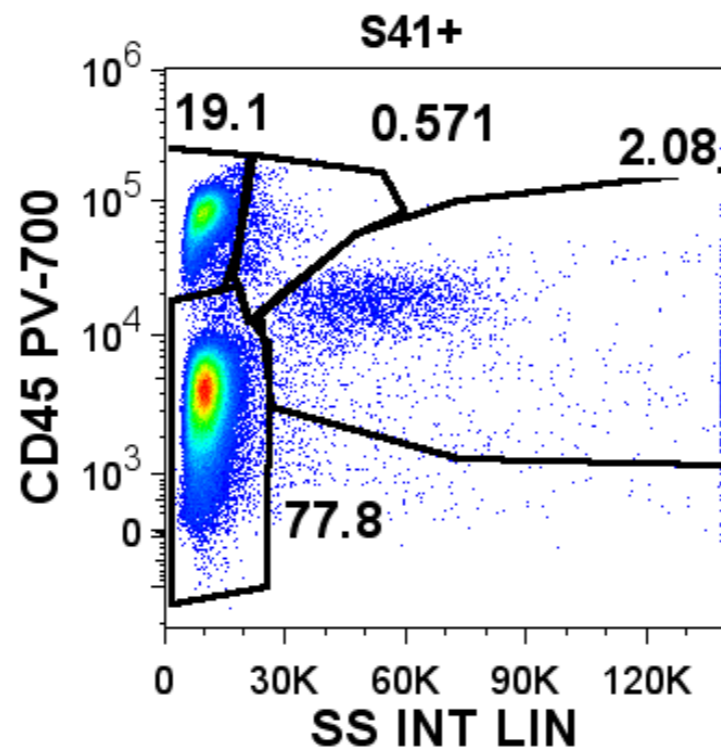
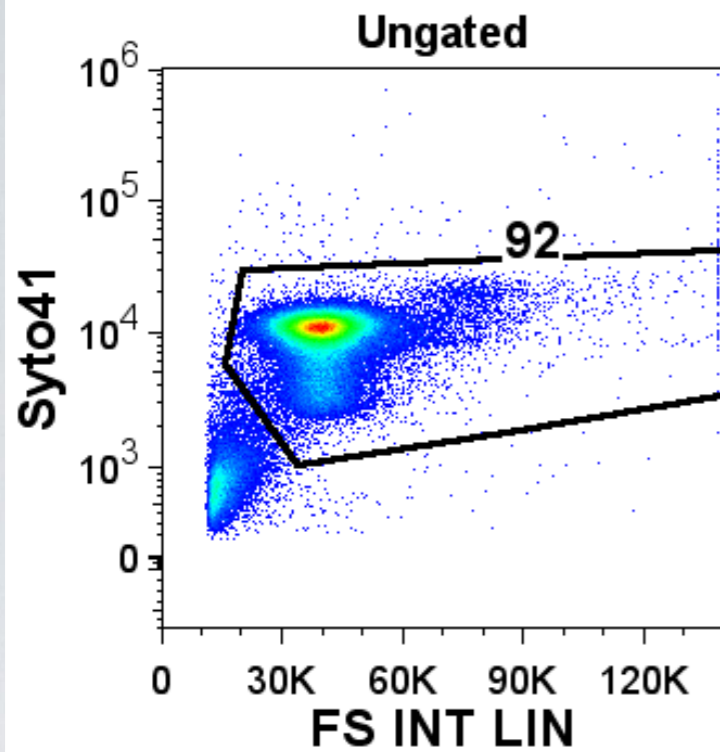
MRD: 10%

# B-ALL MRD, 33. nap



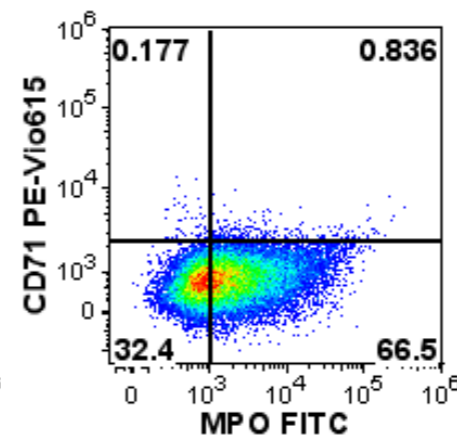
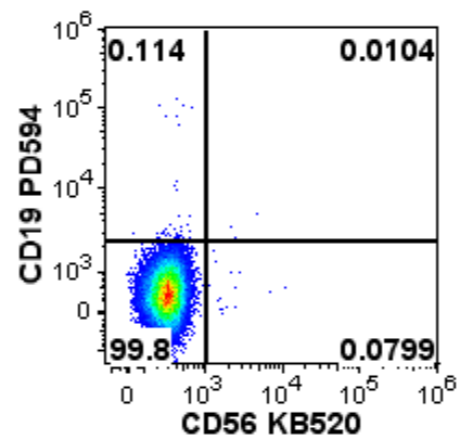
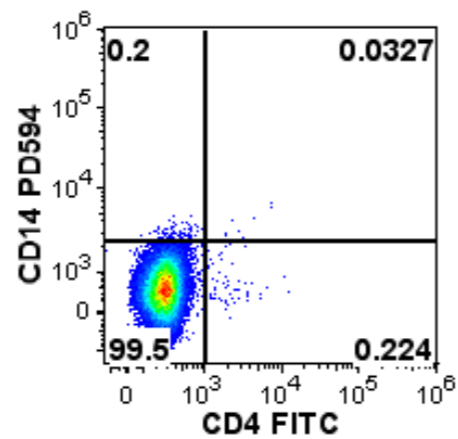
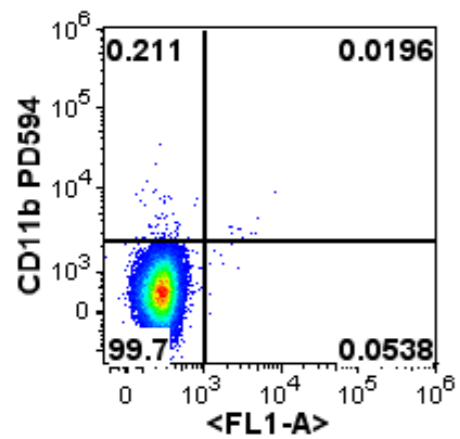
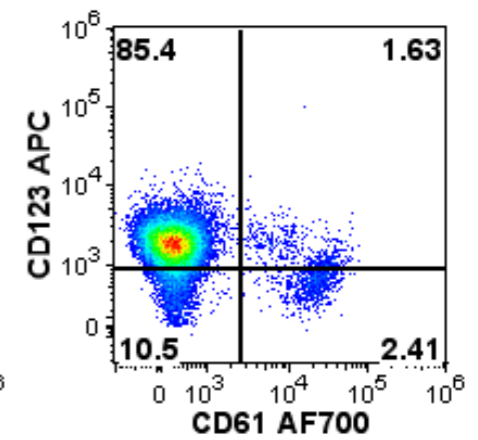
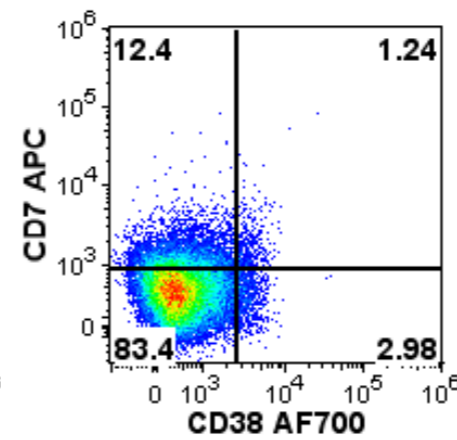
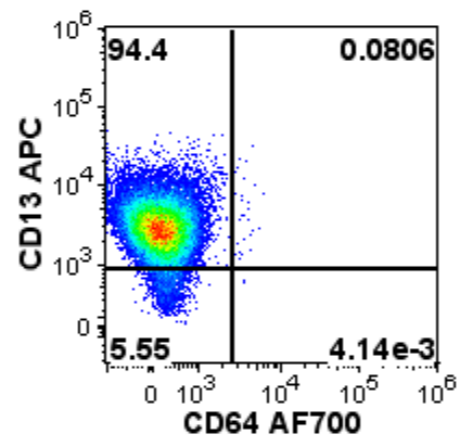
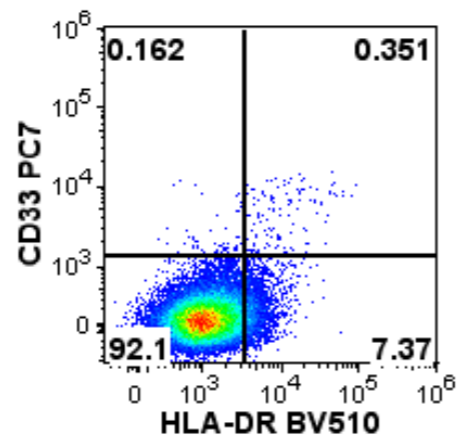
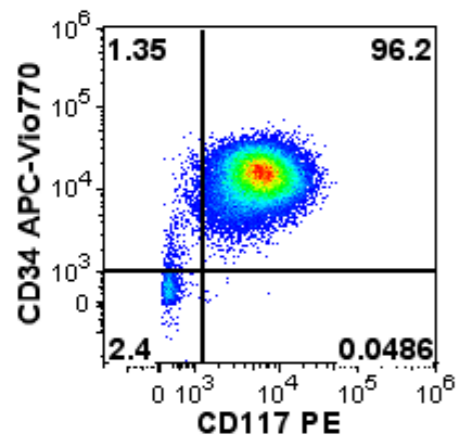
MRD nem igazolható.



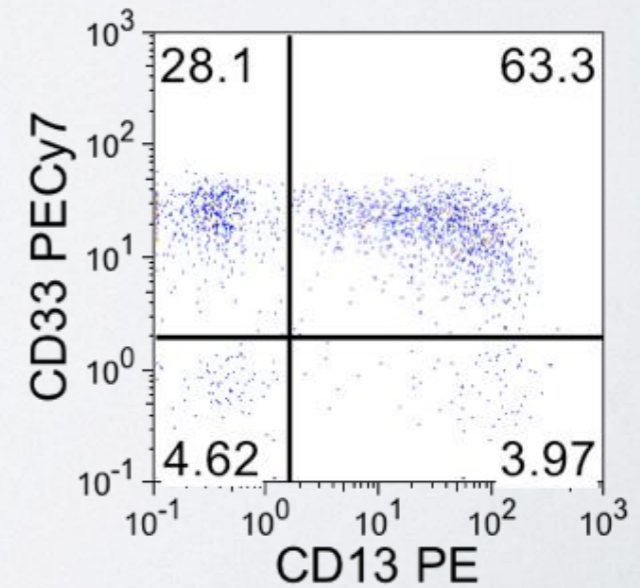
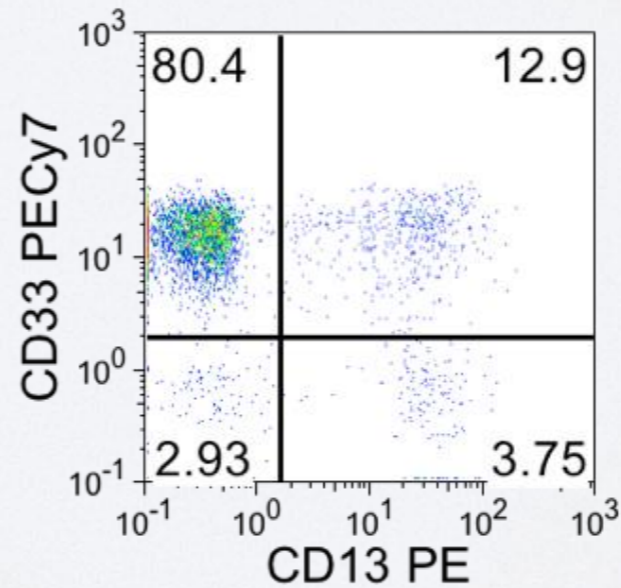
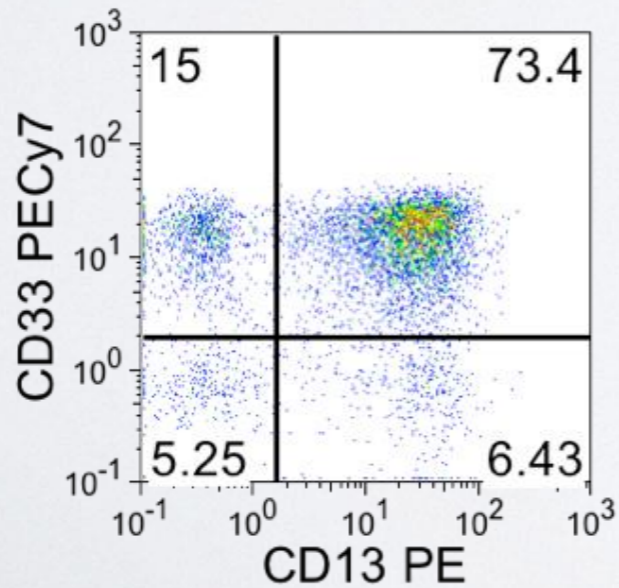
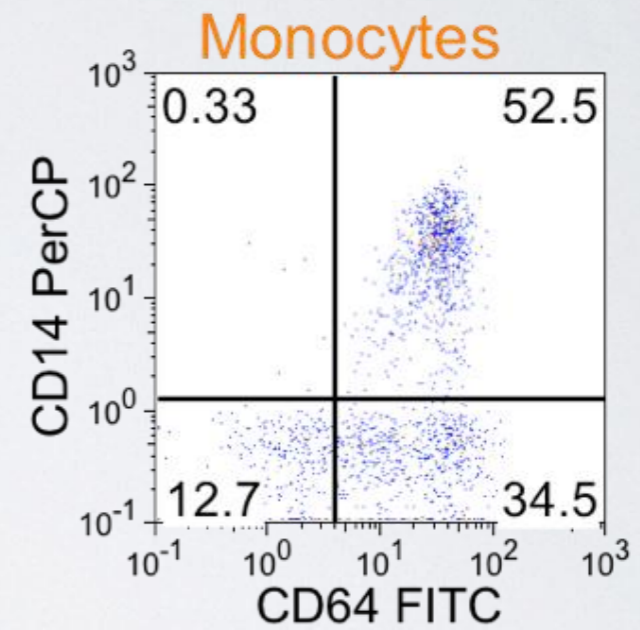
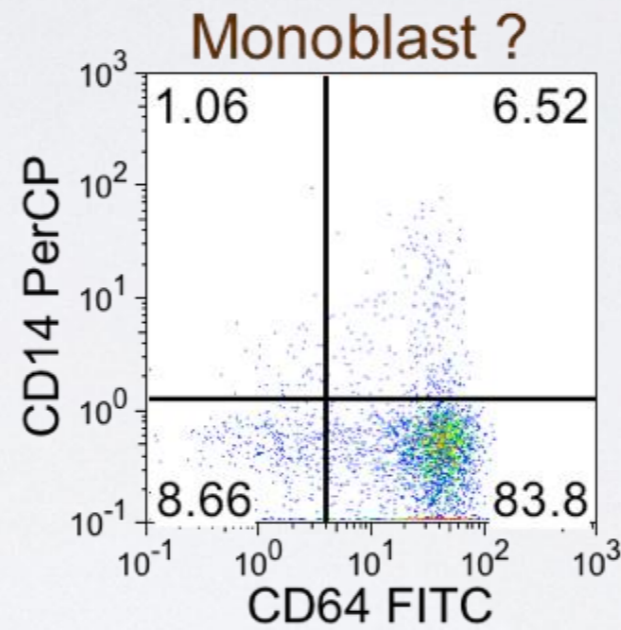
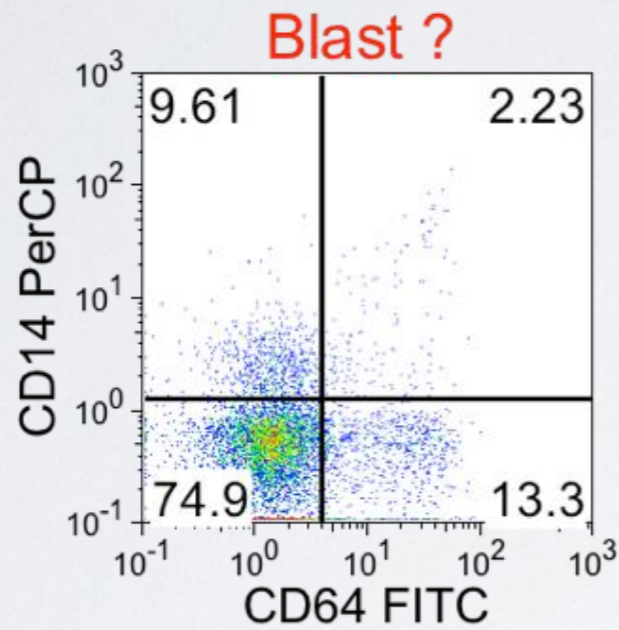
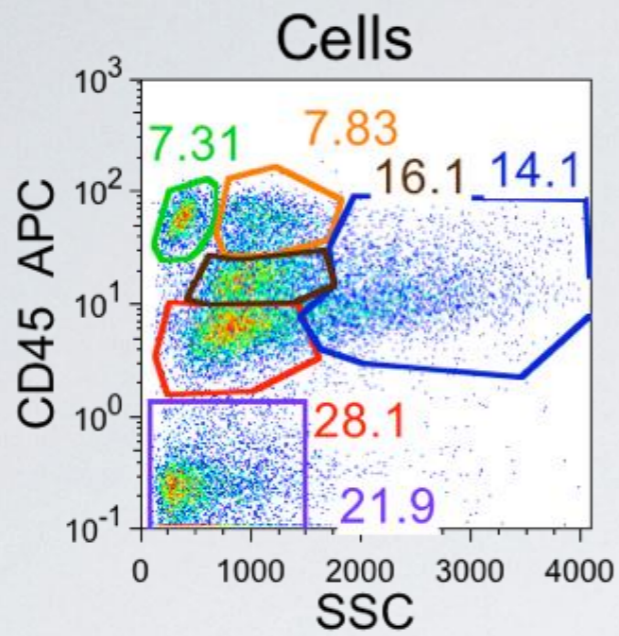


**AML-M0-M1**

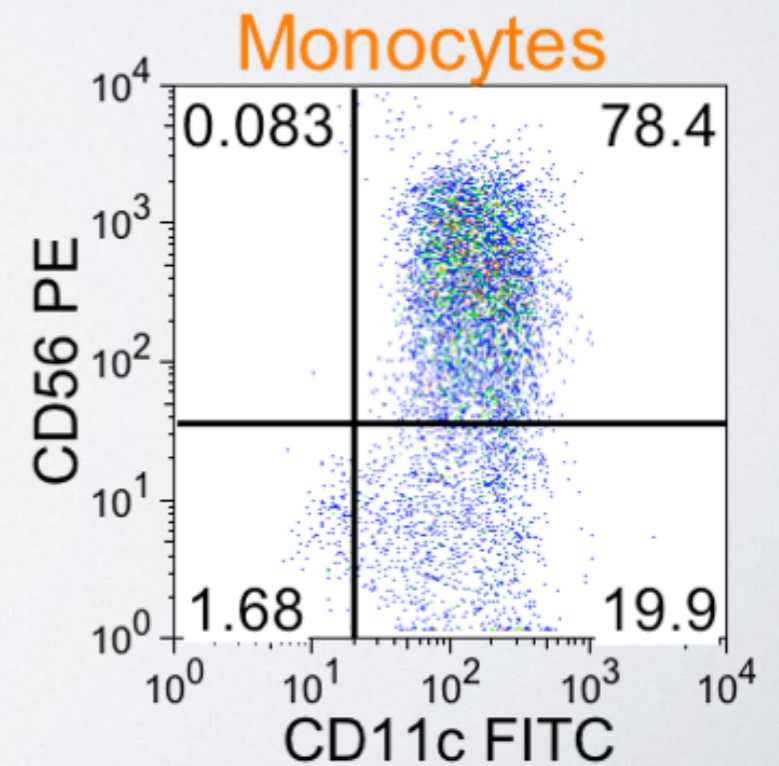
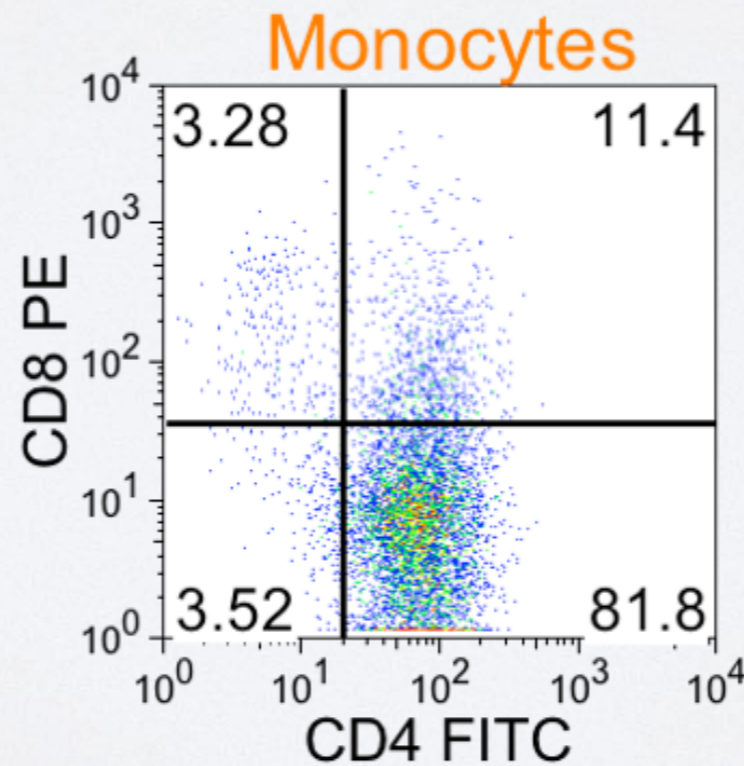
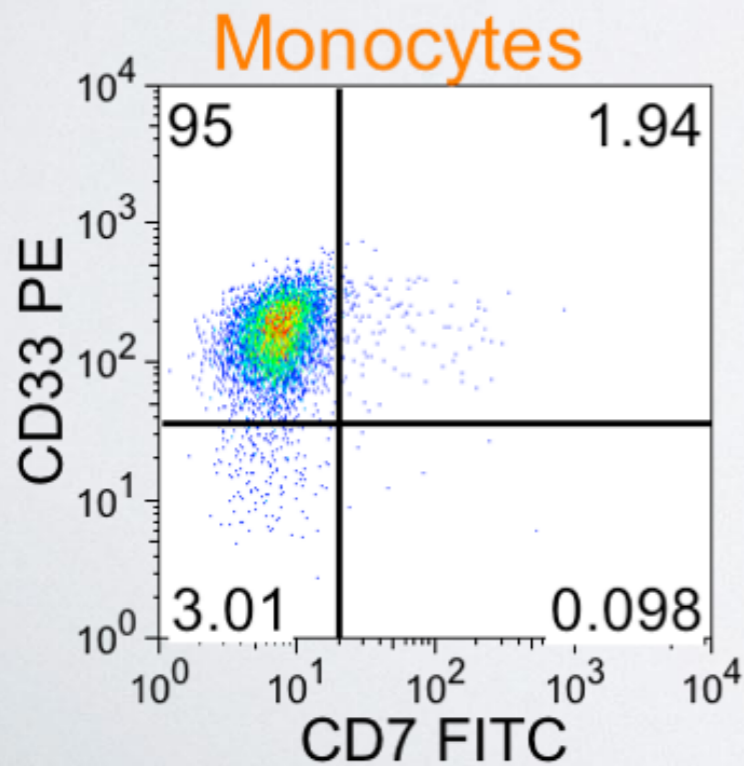
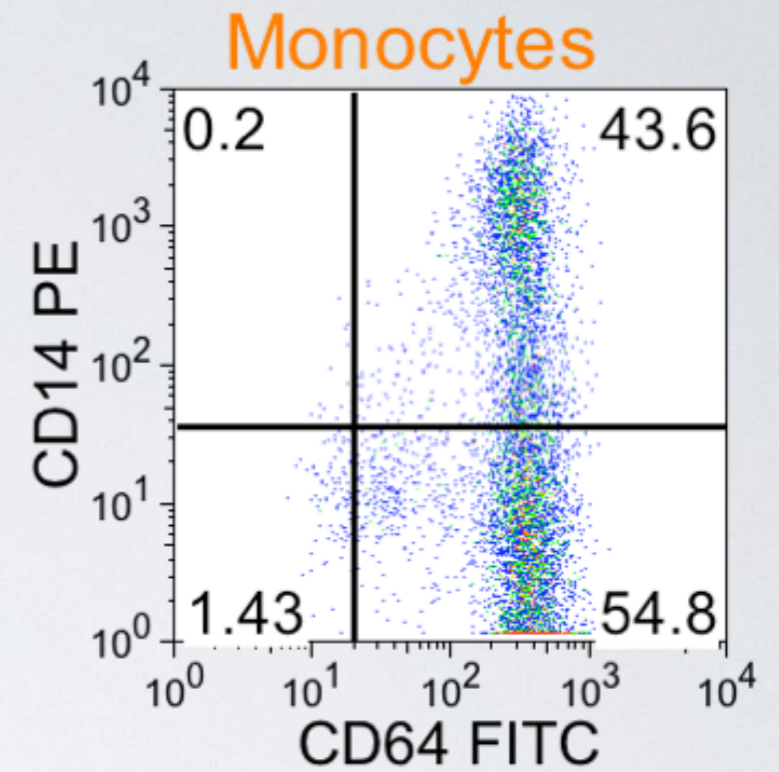
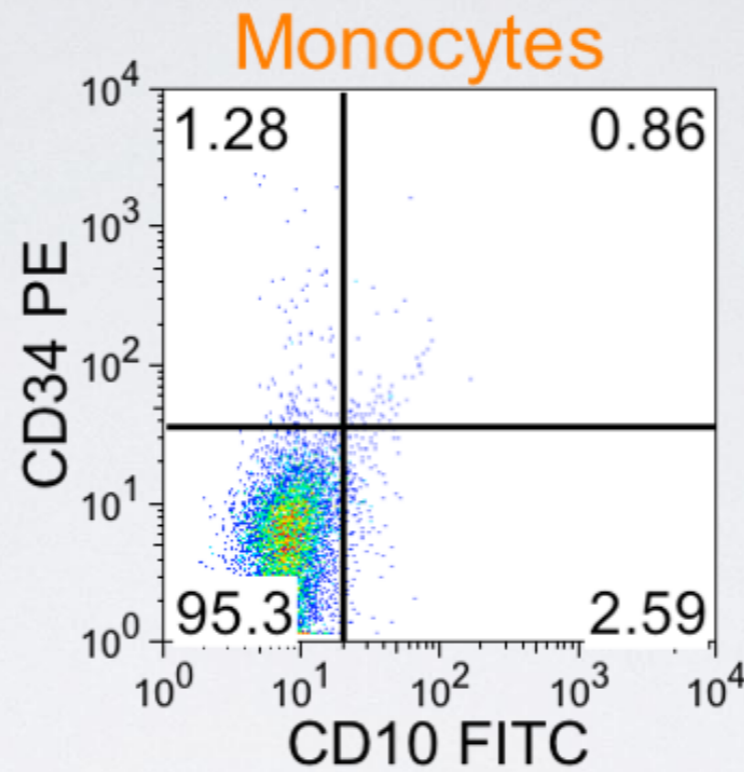
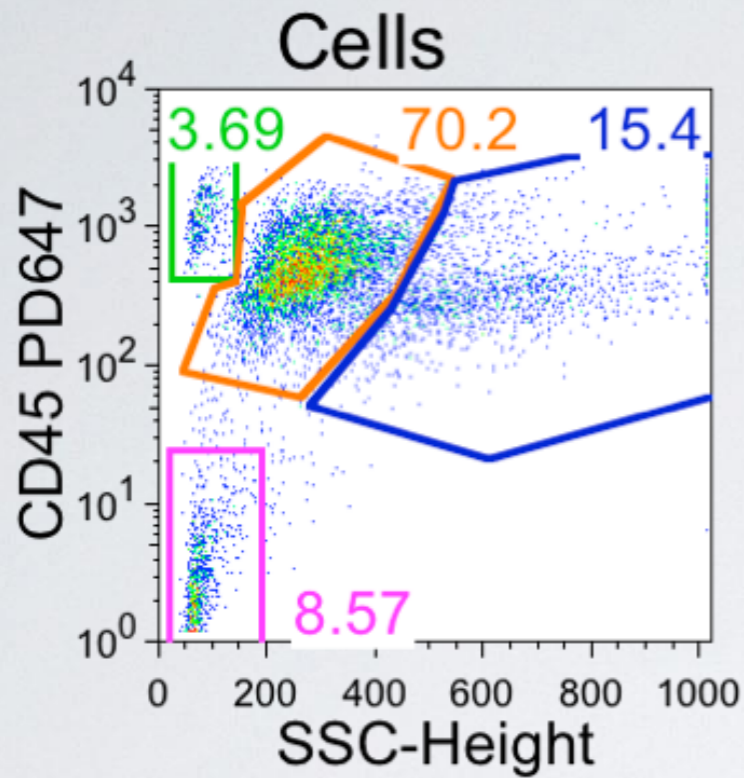
**Blast**



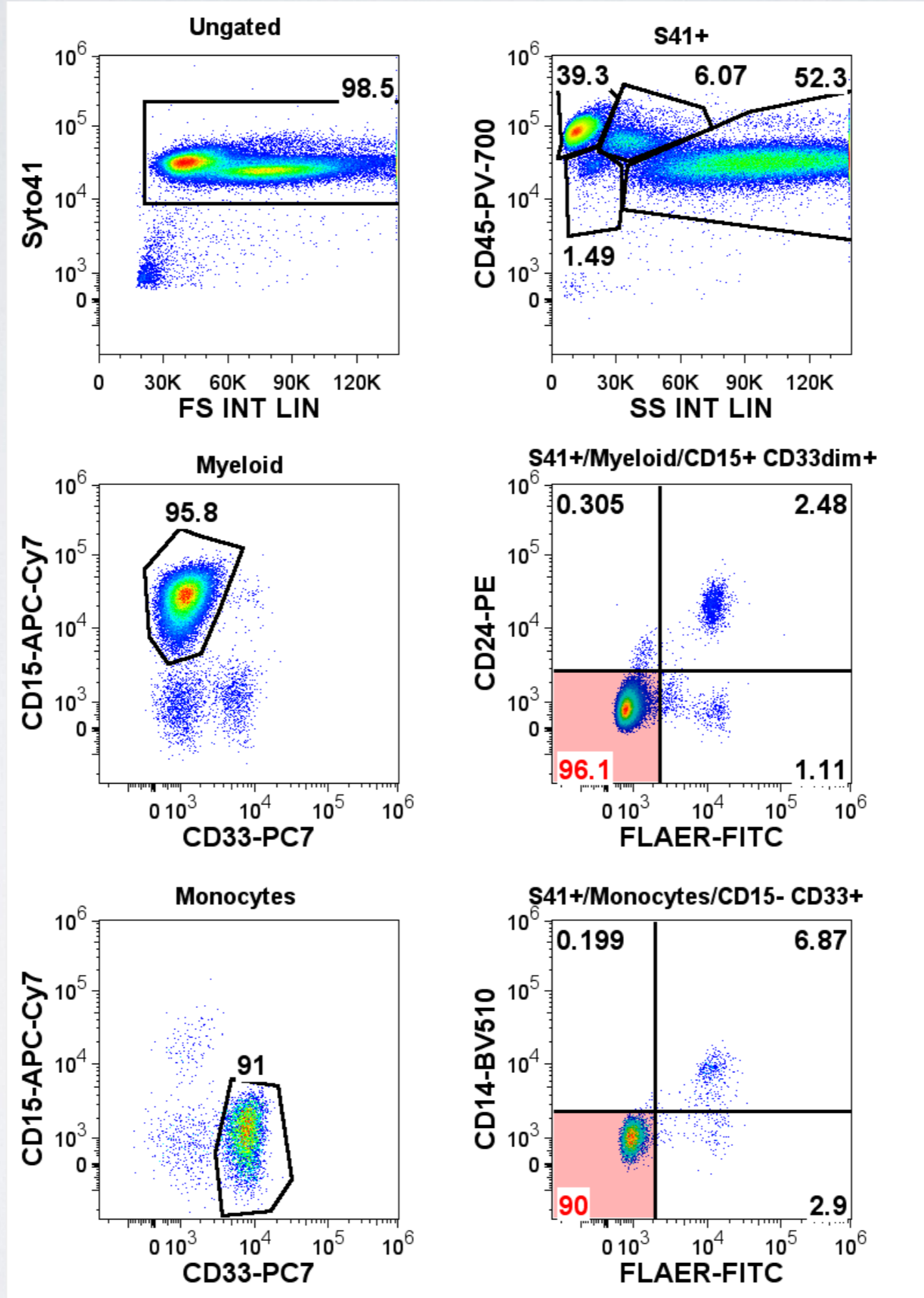
# AML-M4, csontvelő



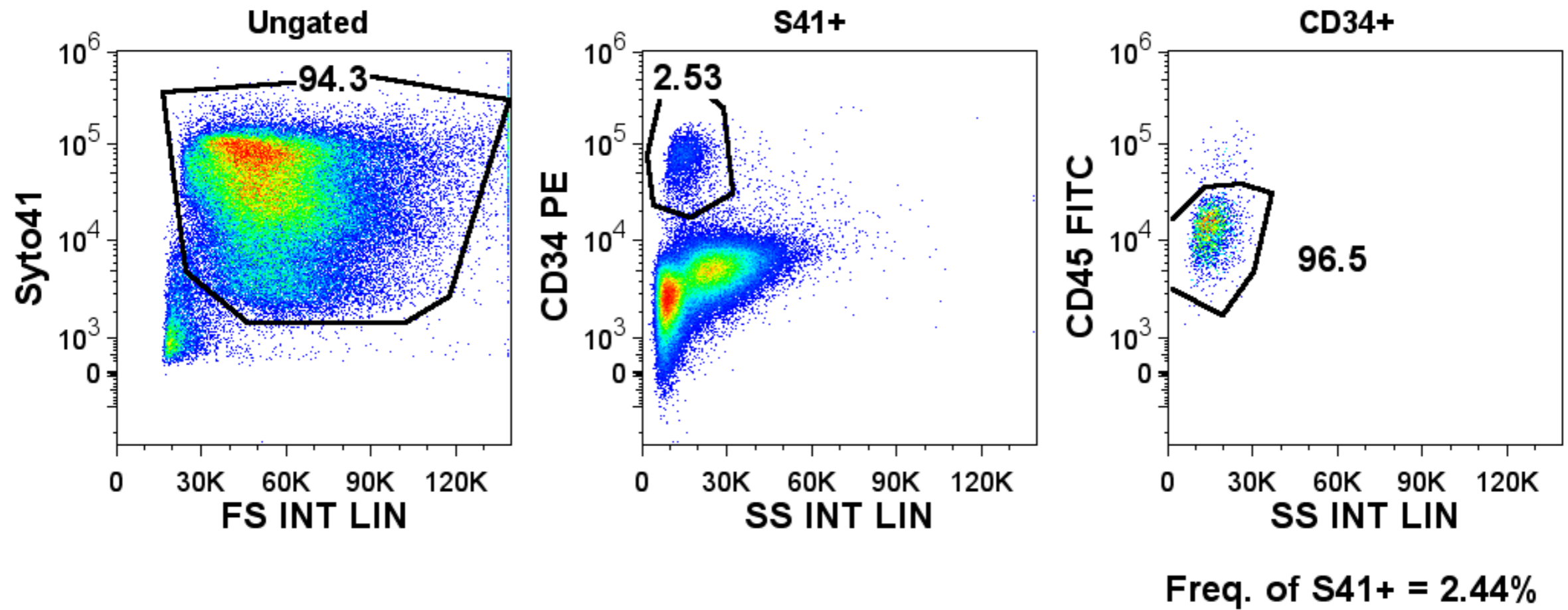
# AML-M5, csontvelő



# PNH



# CD34+ őssejt koncentráció meghatározás



**Köszönöm a figyelmet!**