Clinical Flow Cytometry for the Perplexed

Part 1: Introduction to Gating

David P. Ng, MD

Assistant Clinical Professor, University of Utah School of Medicine

ARUP LABORATORIES

HEALTH UNIVERSITY OF UTAH

Introduction

- 1st of a 5 part series of lectures
  - » Gating
  - » Technical Artifacts
  - » Lymphomas
  - » Myeloid Neoplasms
  - » Minimal Residual Disease

Goals of this Lecture

- Define the parameters used in finding populations of interest
- Understand basic gating strategies used in clinical flow cytometry
- Recognize the difference between gating and phenotyping markers
- Identify ‘junk’ events and ignore them for the purposes of clinical immunophenotyping

Why do you care about flow cytometry?

1. Reduces the differential diagnosis
2. Fast Turn-around-time (<6 hrs)
3. Prognostication and Theranostics
    1. CD49d – CLL
    2. CD20, CD19, CD22, CD33, CD38, CD30…
4. Low level disease
    1. Minimal Residual Disease (MRD)
    2. Monoclonal B cell lymphocytosis (MBL)

What is flow cytometry?

- An interesting combination of:
  - » High voltage (PMTs)
  - » Blinding Lasers (100+ mW lasers)
  - » Saline Pumps (Sheath/Flow Cell fluidics)

- A way to determine the expression of multiple proteins on the surface of individual cells

Why does this matter?

Neoplasm – new (abnormal) growth of cells

- All neoplasm start with a single cell
- Neoplastic cells are clonally related*
- Phenotypes are based on cell of origin* and typically deranged

- TL;DR Neoplastic populations have different phenotypes from normal populations

How do we define populations?

- What do we have to use?
  - » Forward Scatter
  - » Side Scatter
  - » (CD45)
  - » Other markers?

FIRST RULE OF CLINICAL FLOW:
Do not immunophenotype junk

- Debris binds antibody and fluorophores non-specifically
- Debris can be made up of disparate populations
- A lot of blood, sweat, and tears have been shed while phenotyping junk

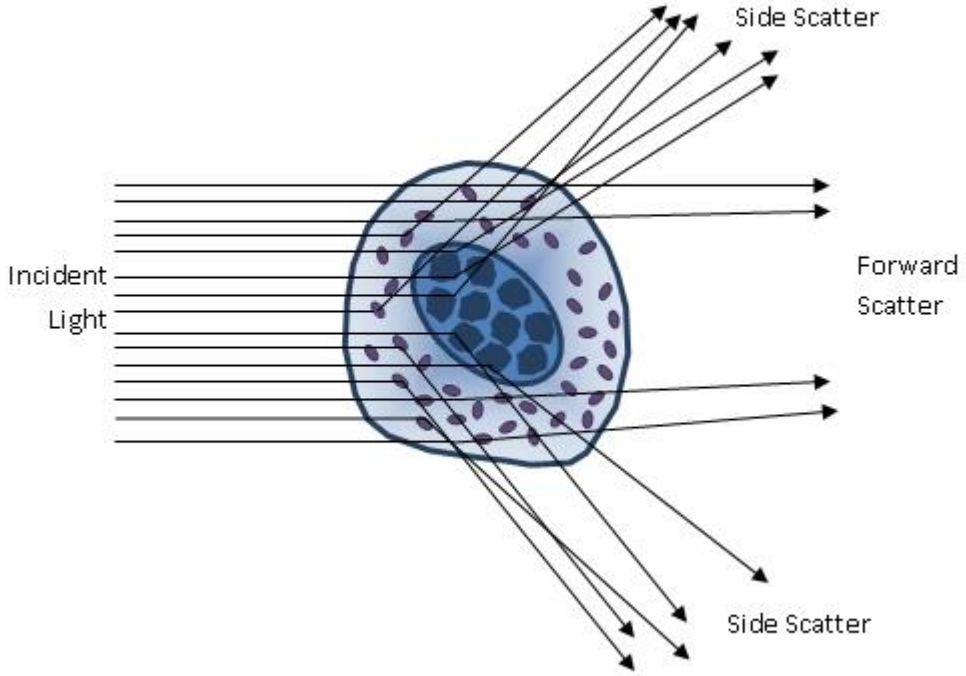
Initial Gating Strategy

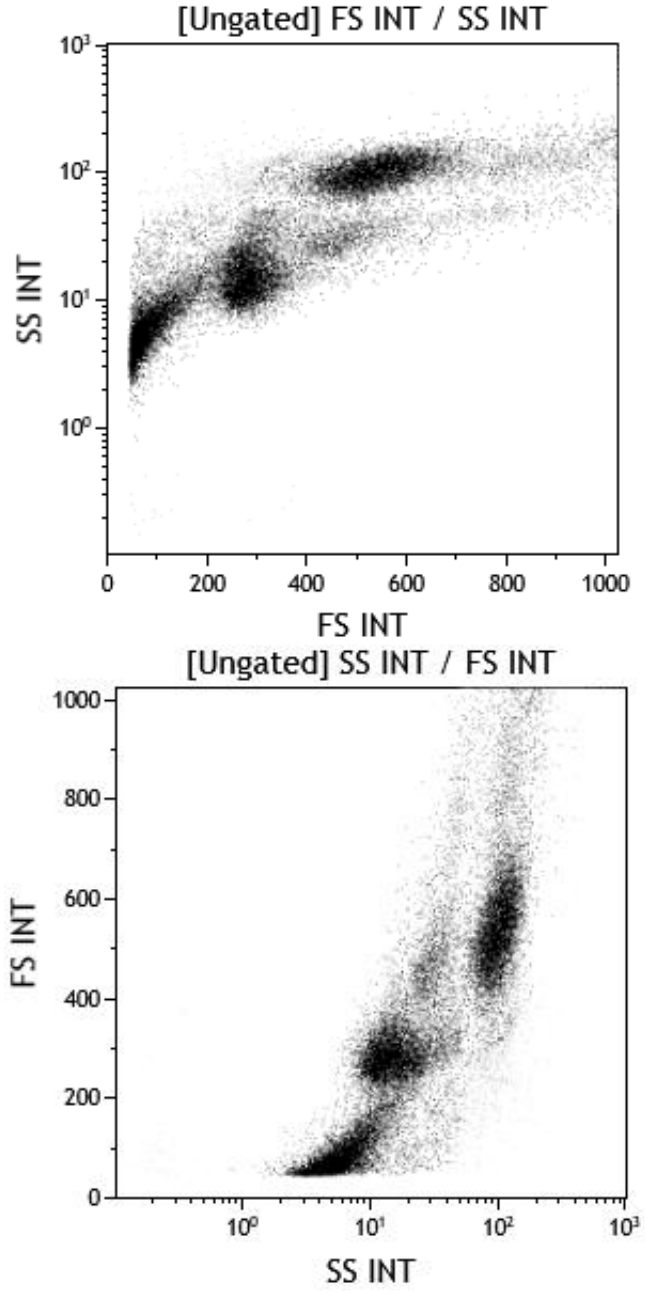
1. Time Gating (filter out clogged events)
2. Doublet Exclusion
3. FS/SS Viability Gating
    - » Potentially Lymph, Mono, Gran Separation

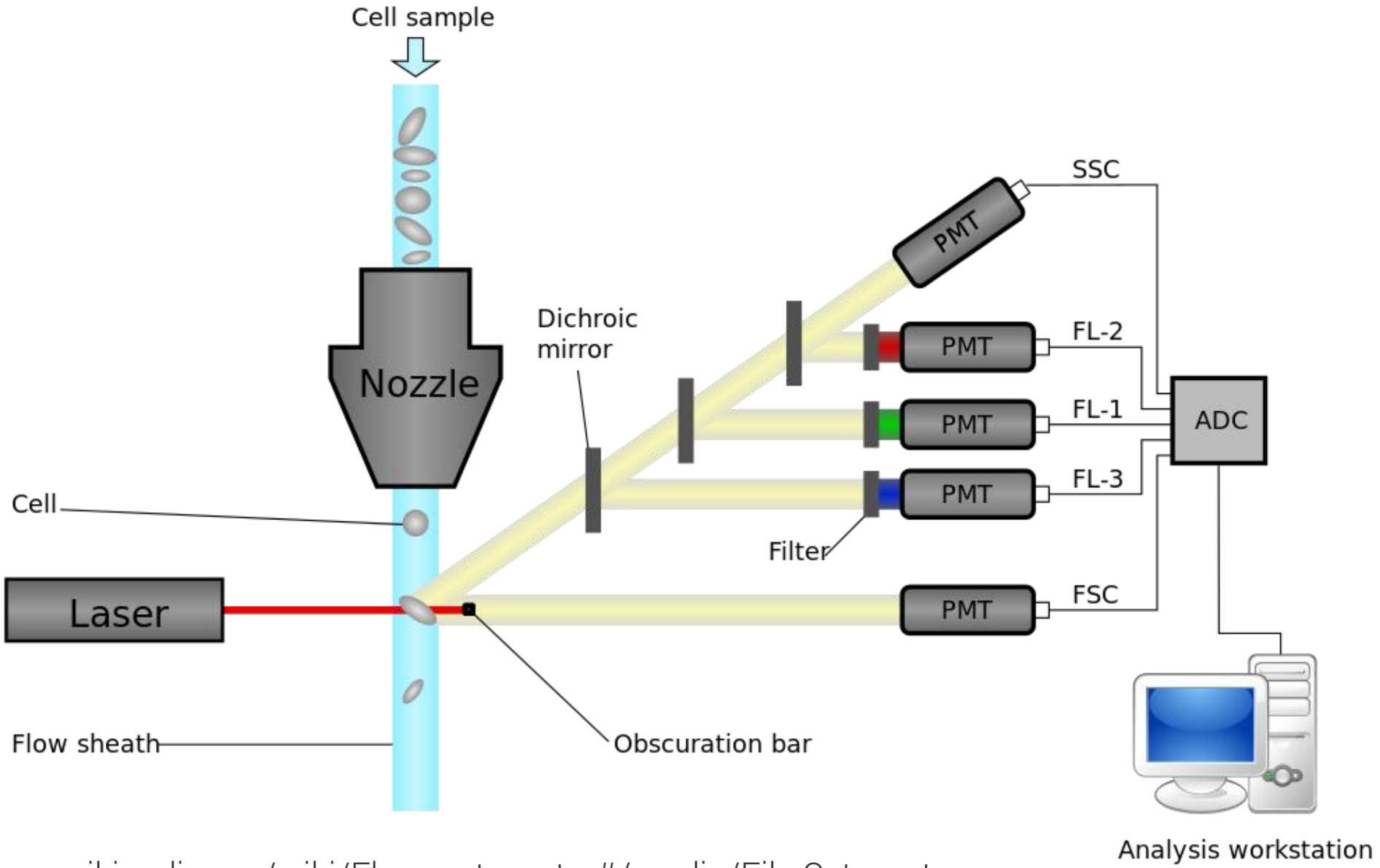
FIRST RULE OF CLINICAL FLOW:

Do not immunophenotype junk

Forward and Side Scatter

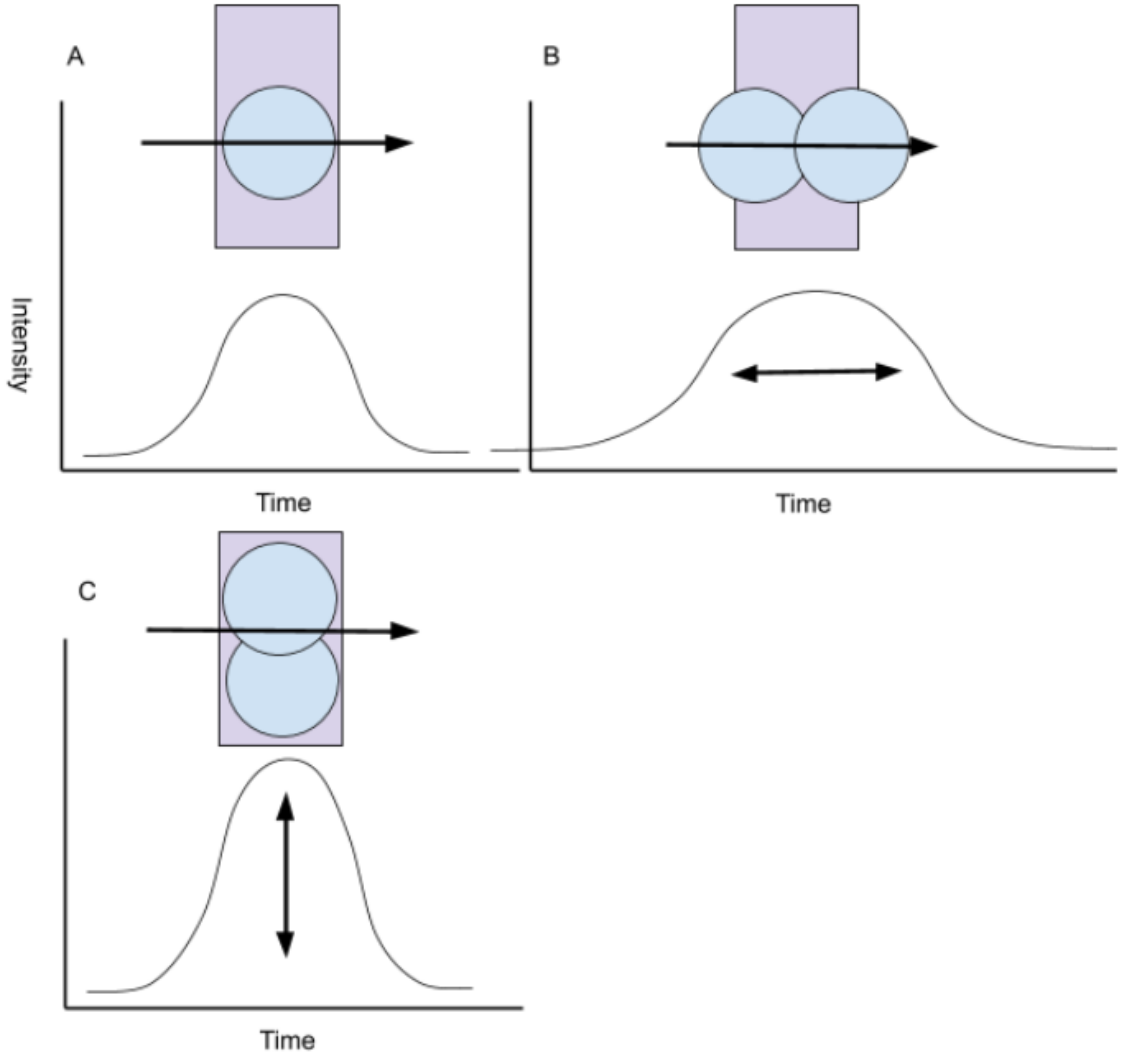


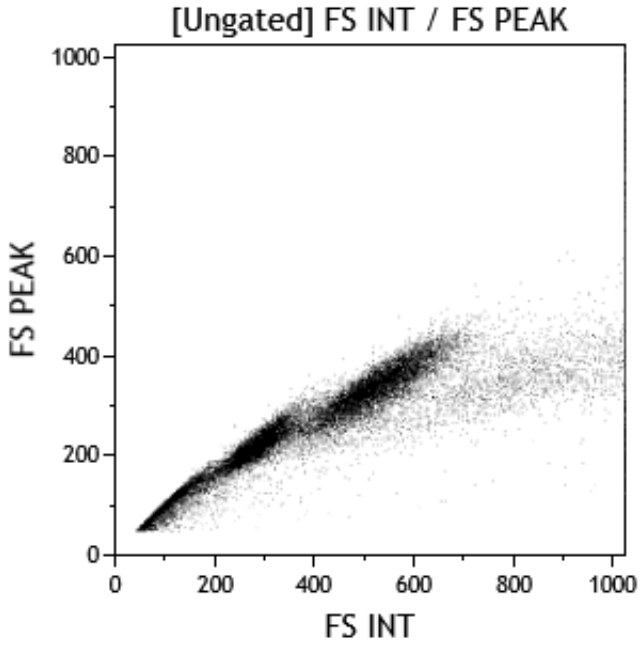




en.wikipedia.org/wiki/Flow_cytometry#/media/File:Cytometer.svg

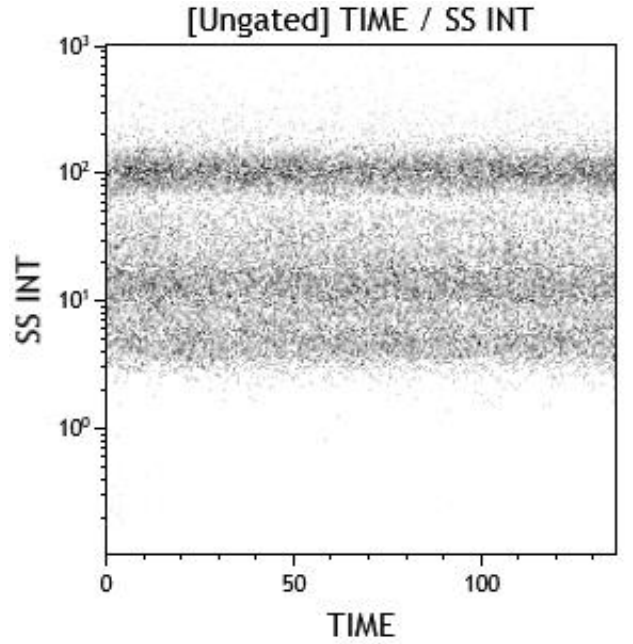
Doublet Exclusion

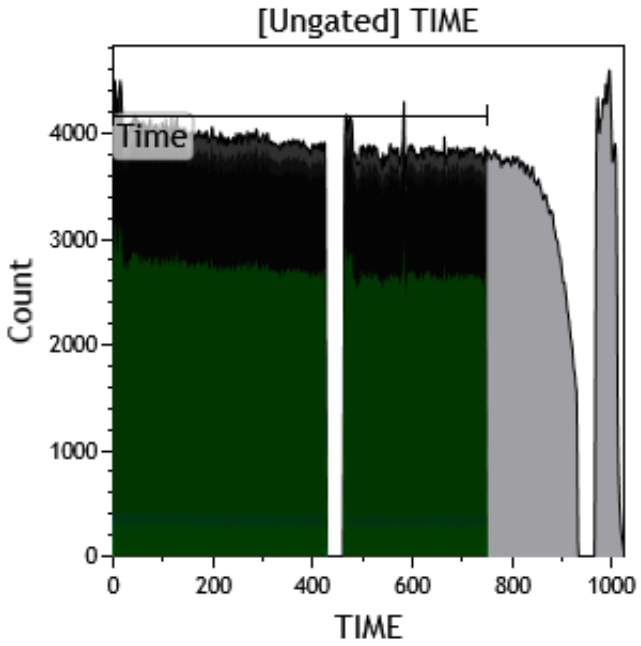




Time Gating

- Clogs happen
- Cells settle (long collections)





Gating vs Phenotyping Reagents

- Some markers are used to define population
- Some markers are used to detect aberrancies
- There is overlap!

Ergo:

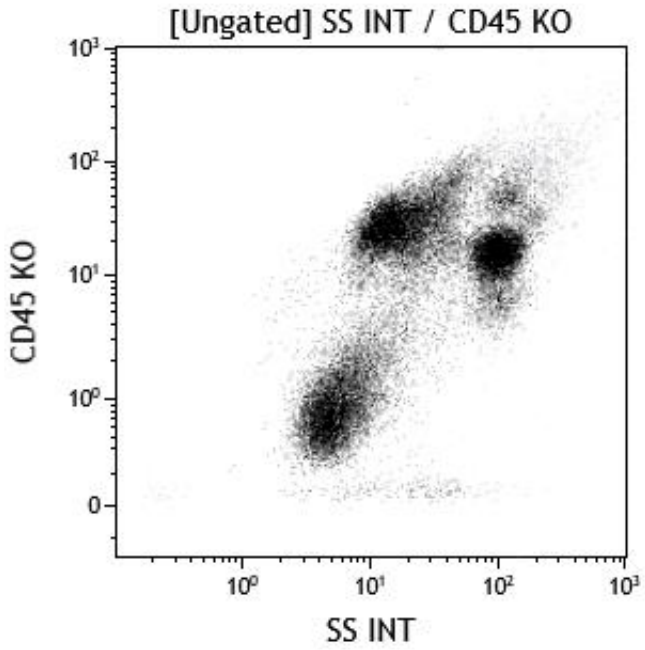
SECOND RULE OF CLINICAL FLOW

If you gate with a marker:

1. You may miss an abnormal population that lost that marker
2. You may not be able to tell it is aberrant

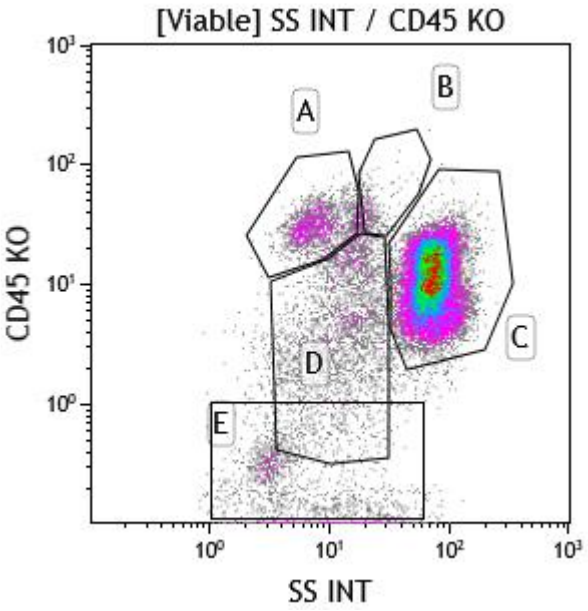
CD45/SS Gating

- Classic Gating Technique
- Advantages
  - » Separates out non-leukocytes
  - » Separates out blasts/immature populations
  - » Red blood cells (should be lysed) are CD45 negative
- Disadvantages
  - » Uses an extra channel
  - » Often redundant



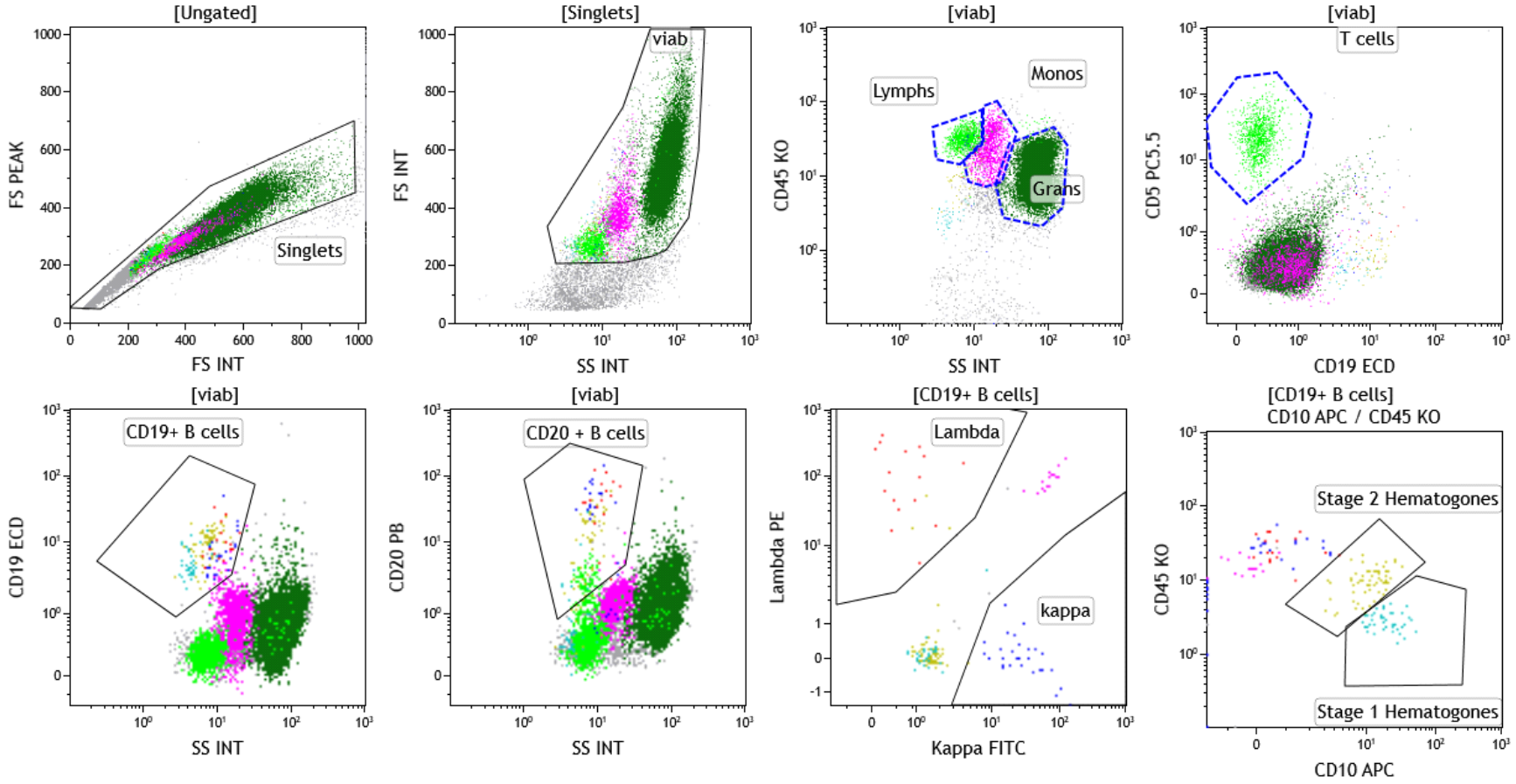
An Example

- CD45/SS gating looking at expression patterns within:
  - A – Lymphocytes
  - B – Monocytes
  - C – Granulocytes
  - D – Blasts
  - E – CD45-low to negative



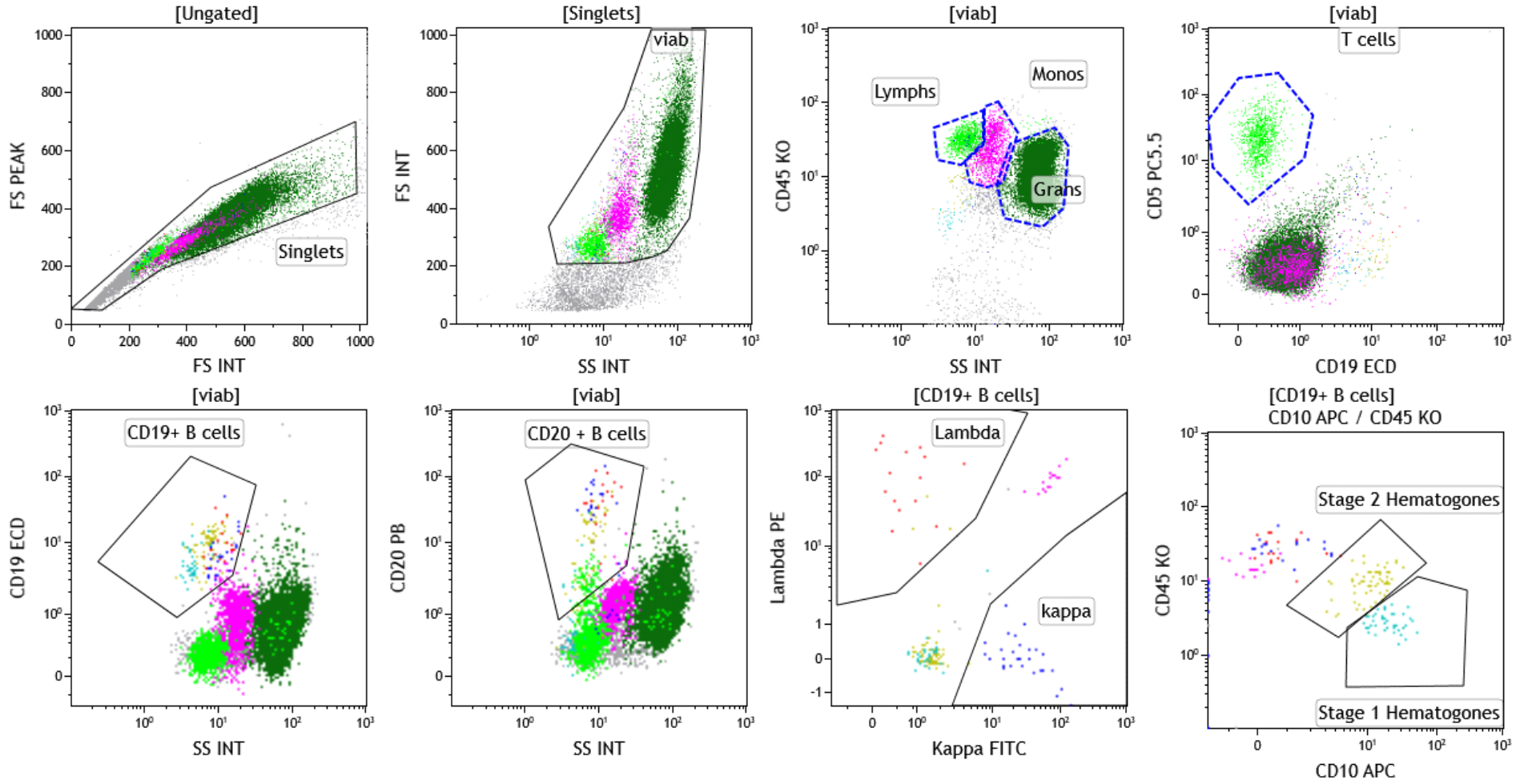
Backgating

- Differentially color populations based on some down-stream gating characteristics

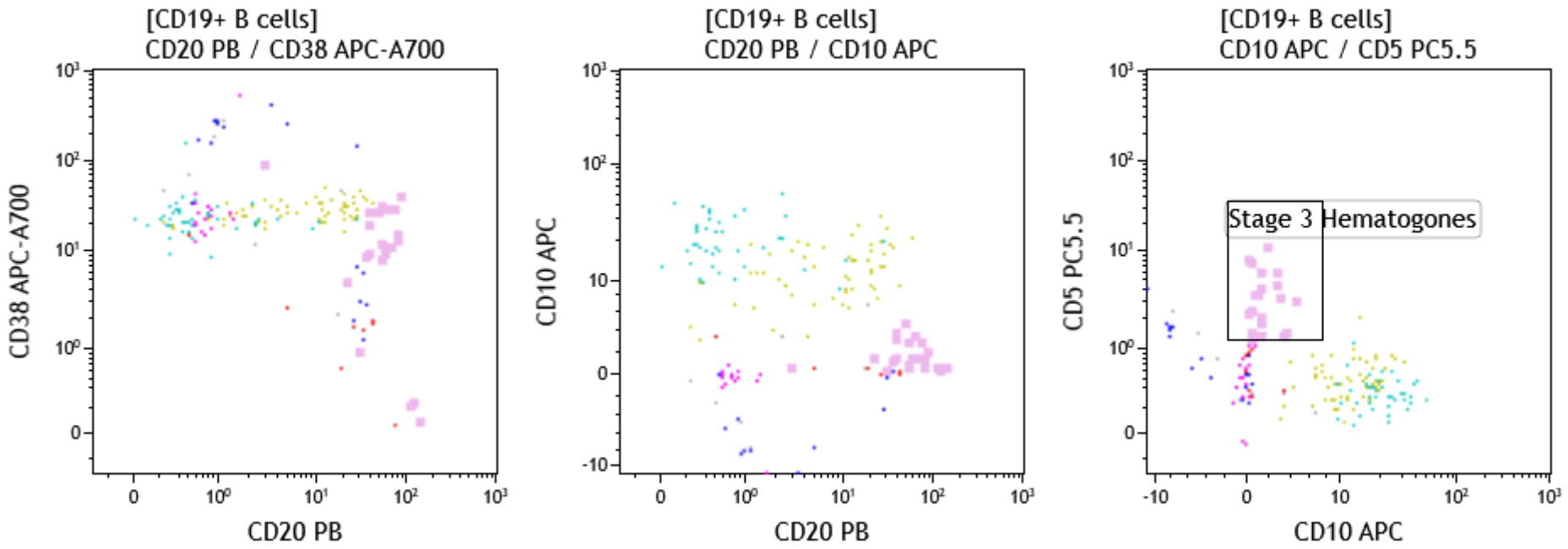


Gating Strategy for B cells

- CD19 AND CD20



What does this buy you?



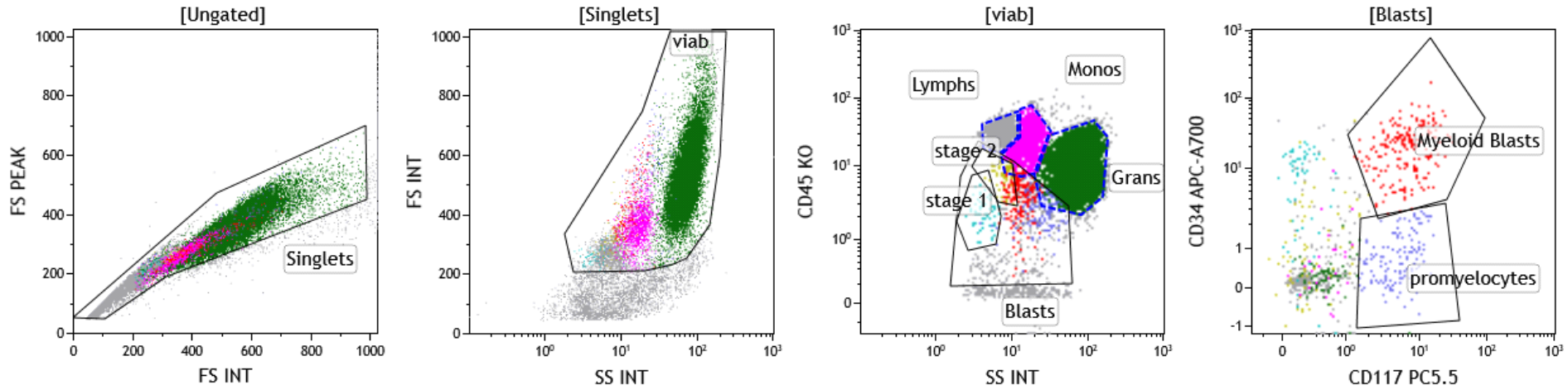
THIRD RULE OF CLINICAL FLOW

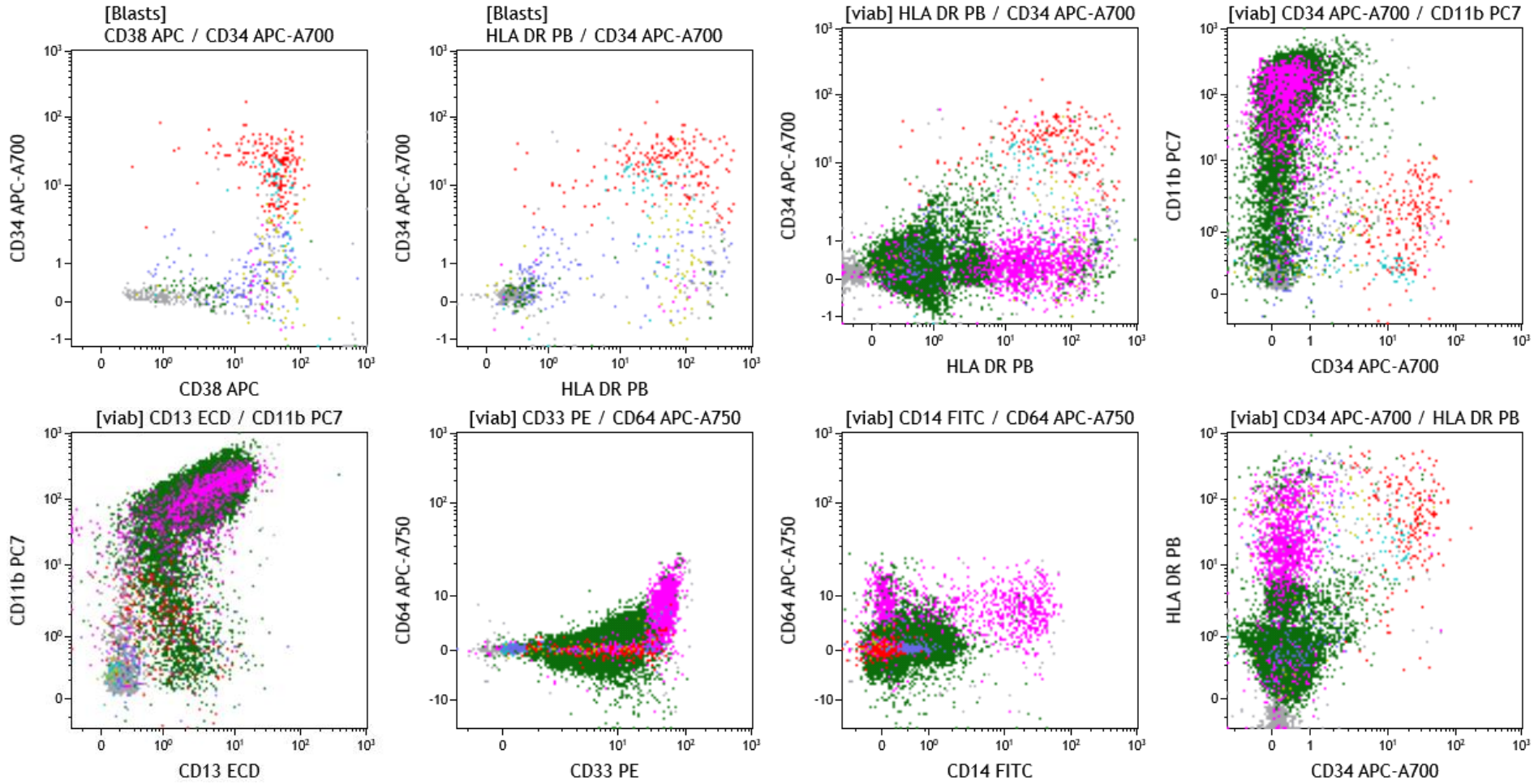
Backgate populations liberally if you want to go home on time.

Gating Strategy for Blasts

- What is a blast?

- CD45/SS
- CD34
- CD117

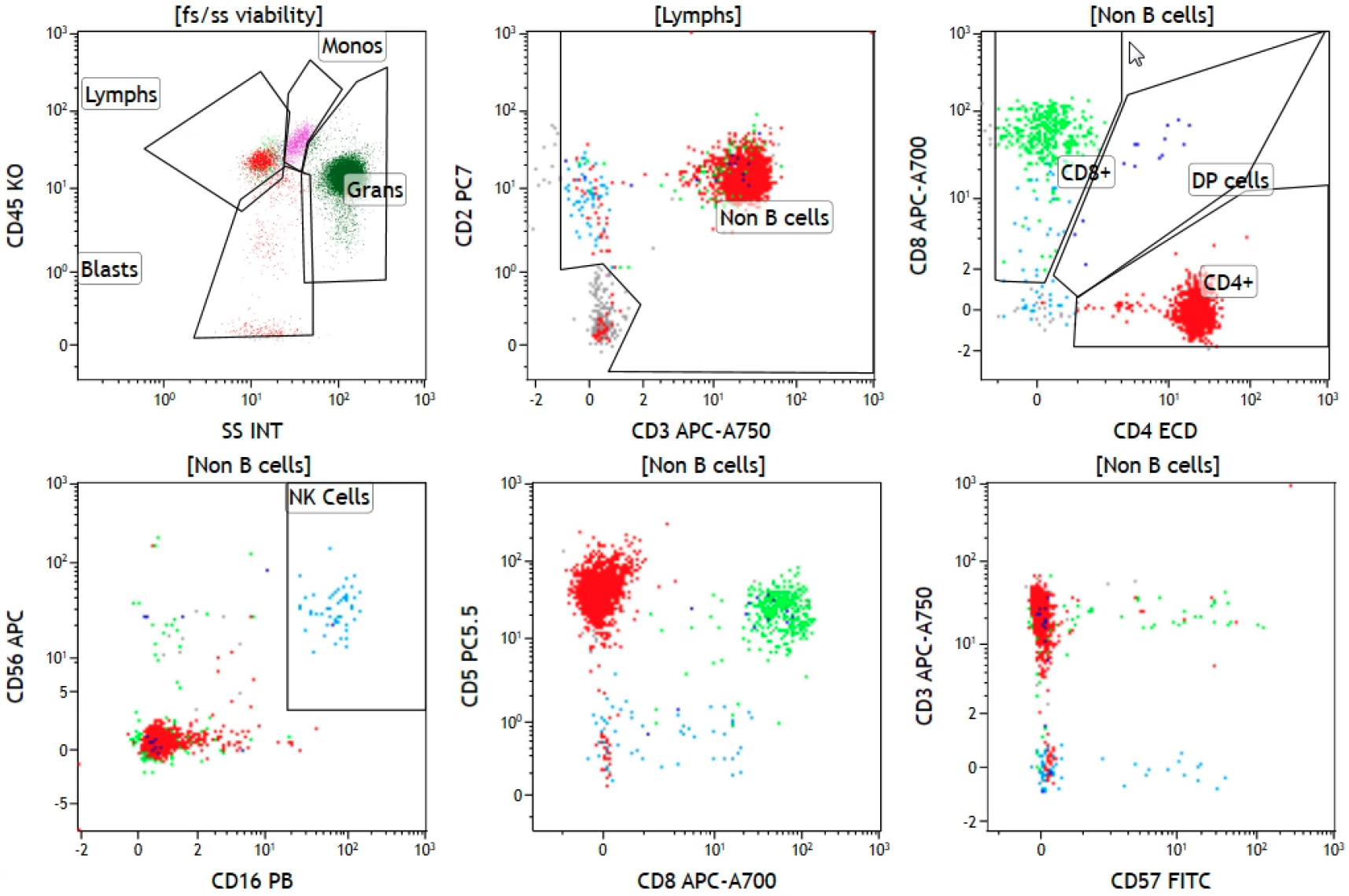

[Ungated]
FS PEAK
FS INT
Singlets
[Singlets]
FS INT
SS INT
viab
[viab]
CD45 KO
SS INT
Lymphs
Monos
stage 2
Grans
stage 1
Blasts
[Blasts]
CD34 APC-A700
CD117 PC5.5
Myeloid Blasts
promyelocytes


[Blasts]
CD38 APC / CD34 APC-A700
CD34 APC-A700
CD38 APC
[Blasts]
HLA DR PB / CD34 APC-A700
CD34 APC-A700
HLA DR PB
[viab] HLA DR PB / CD34 APC-A700
CD34 APC-A700
HLA DR PB
[viab] CD34 APC-A700 / CD11b PC7
CD11b PC7
CD34 APC-A700
[viab] CD13 ECD / CD11b PC7
CD11b PC7
CD13 ECD
[viab] CD33 PE / CD64 APC-A750
CD64 APC-A750
CD33 PE
[viab] CD14 FITC / CD64 APC-A750
CD64 APC-A750
CD14 FITC
[viab] CD34 APC-A700 / HLA DR PB
HLA DR PB
CD34 APC-A700

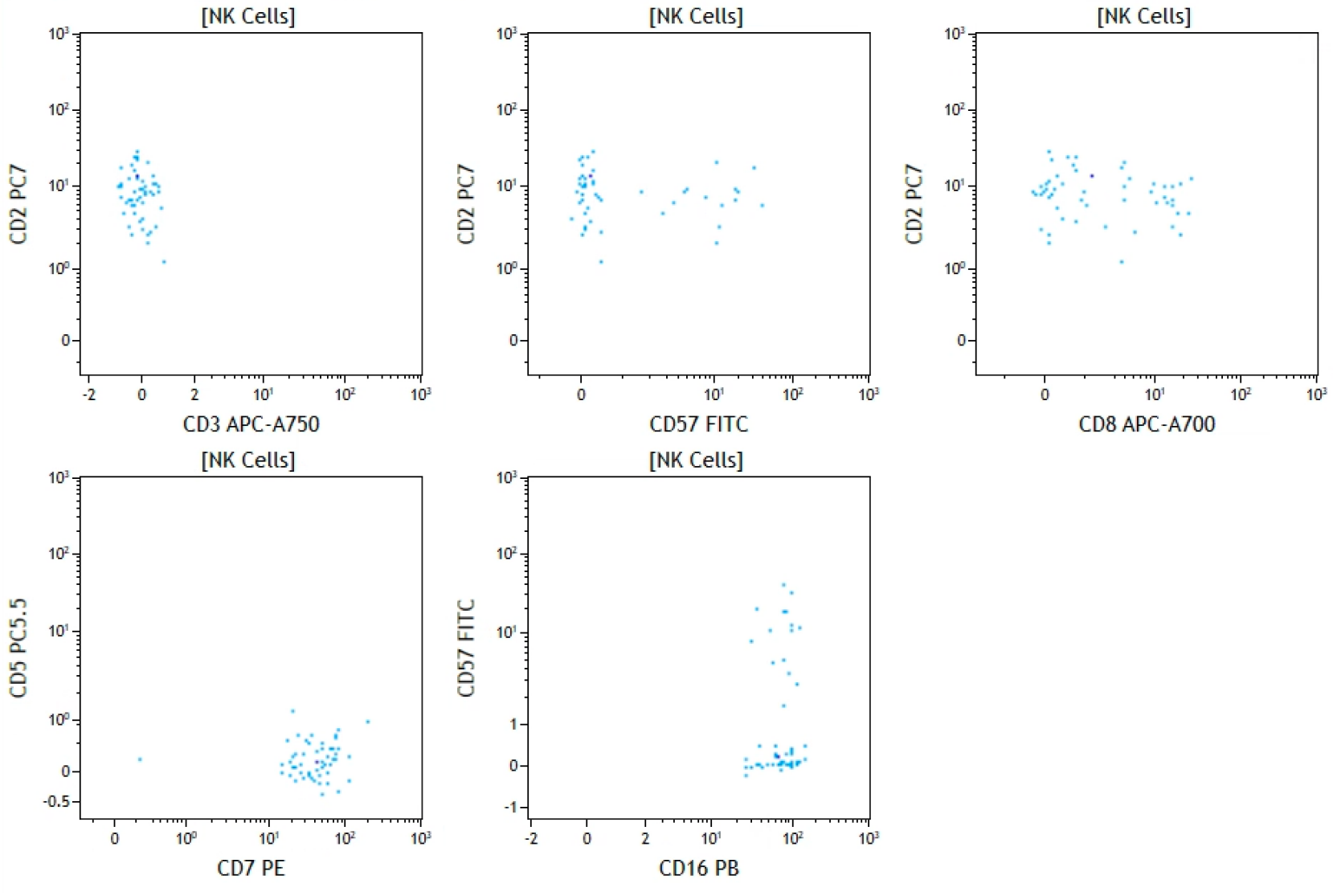
Gating Strategy for T cells

- CD3/CD2/CD5
- Don’t use CD7 (normally lost in a subset of T cells)
- Choose an input gate that maximizes information displayed and minimizes noise
- Note where your gated populations come from (assumptions hidden in the display)

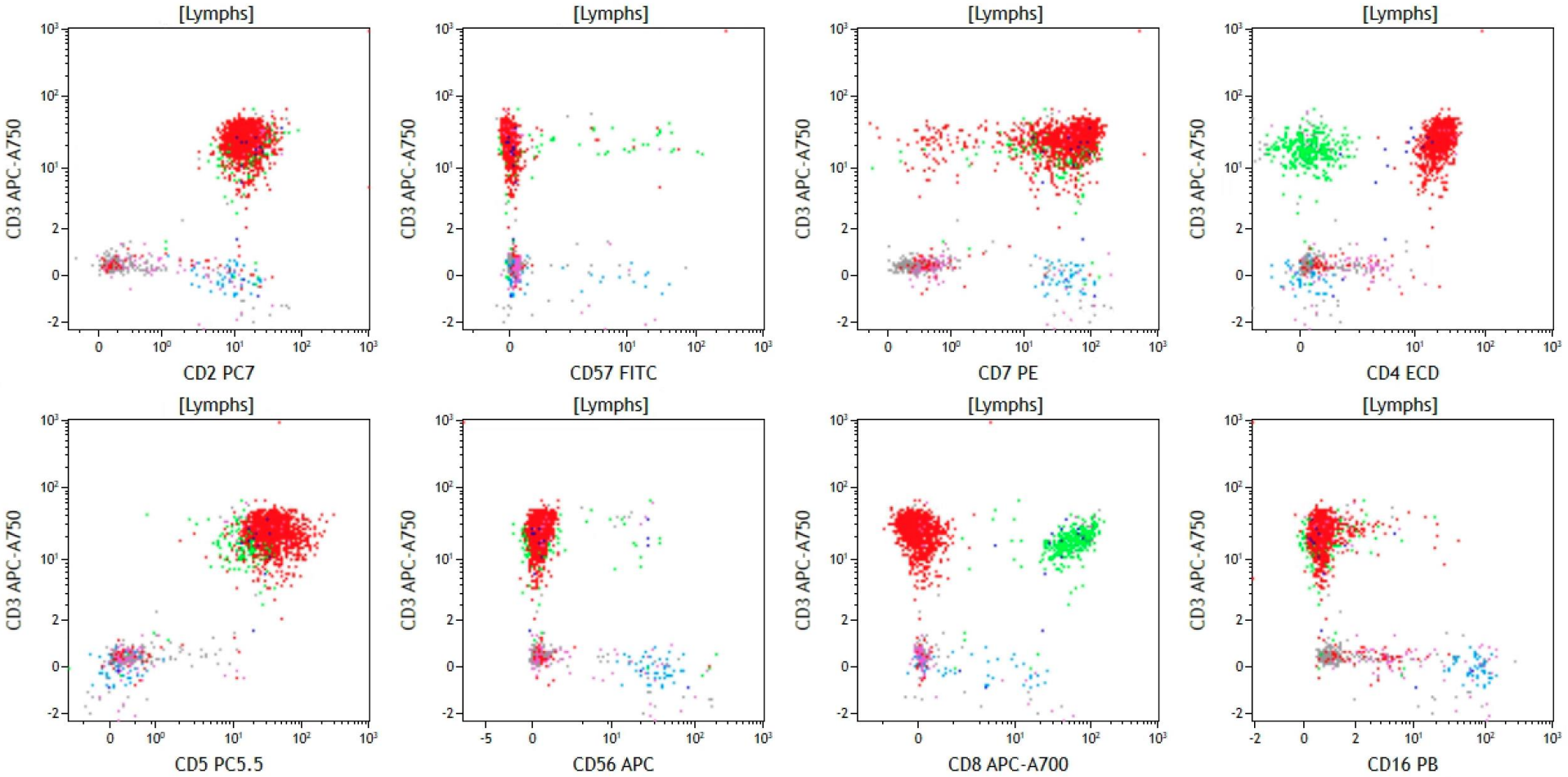
Initial gating for T cells



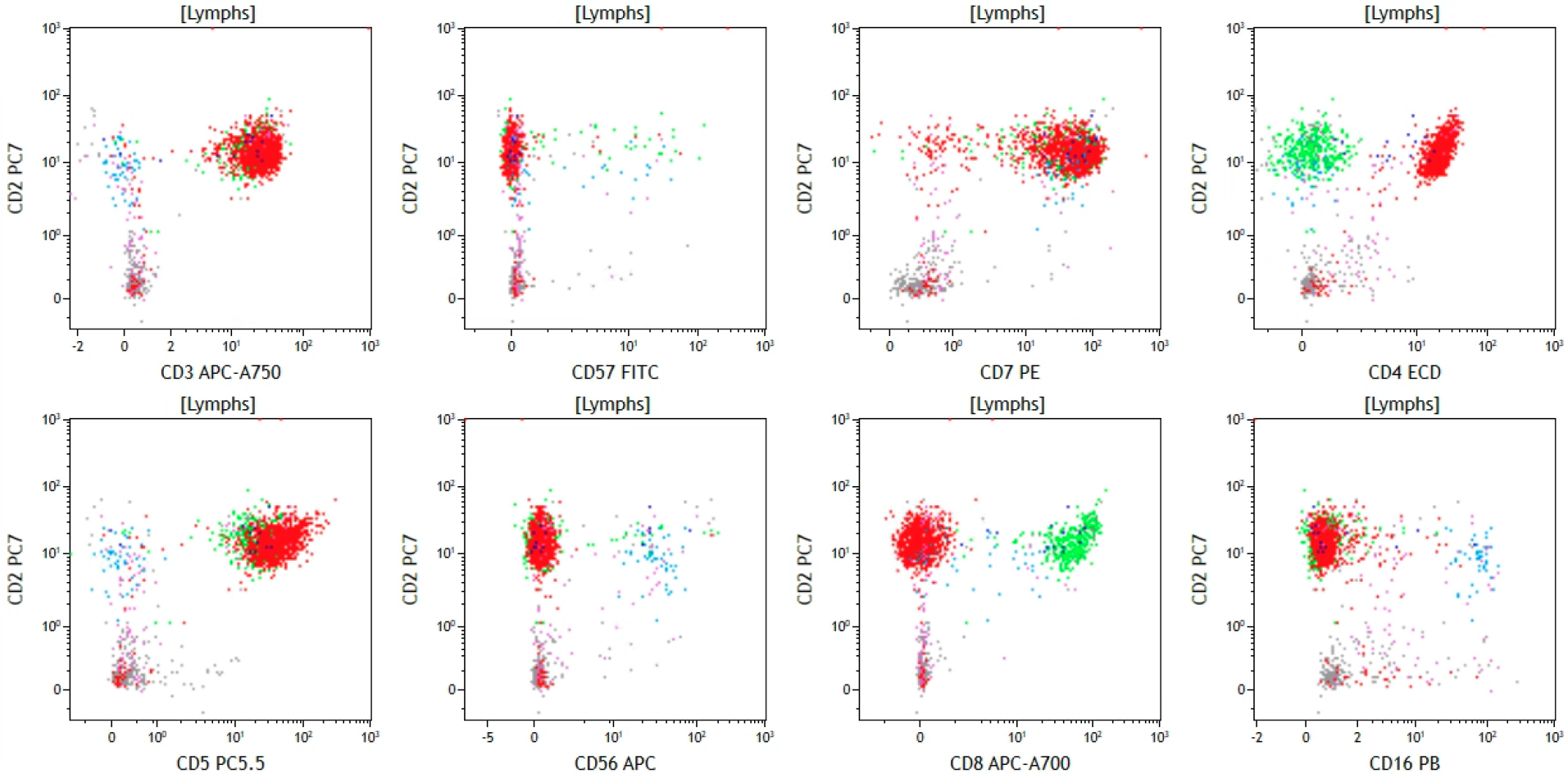
Subpopulations



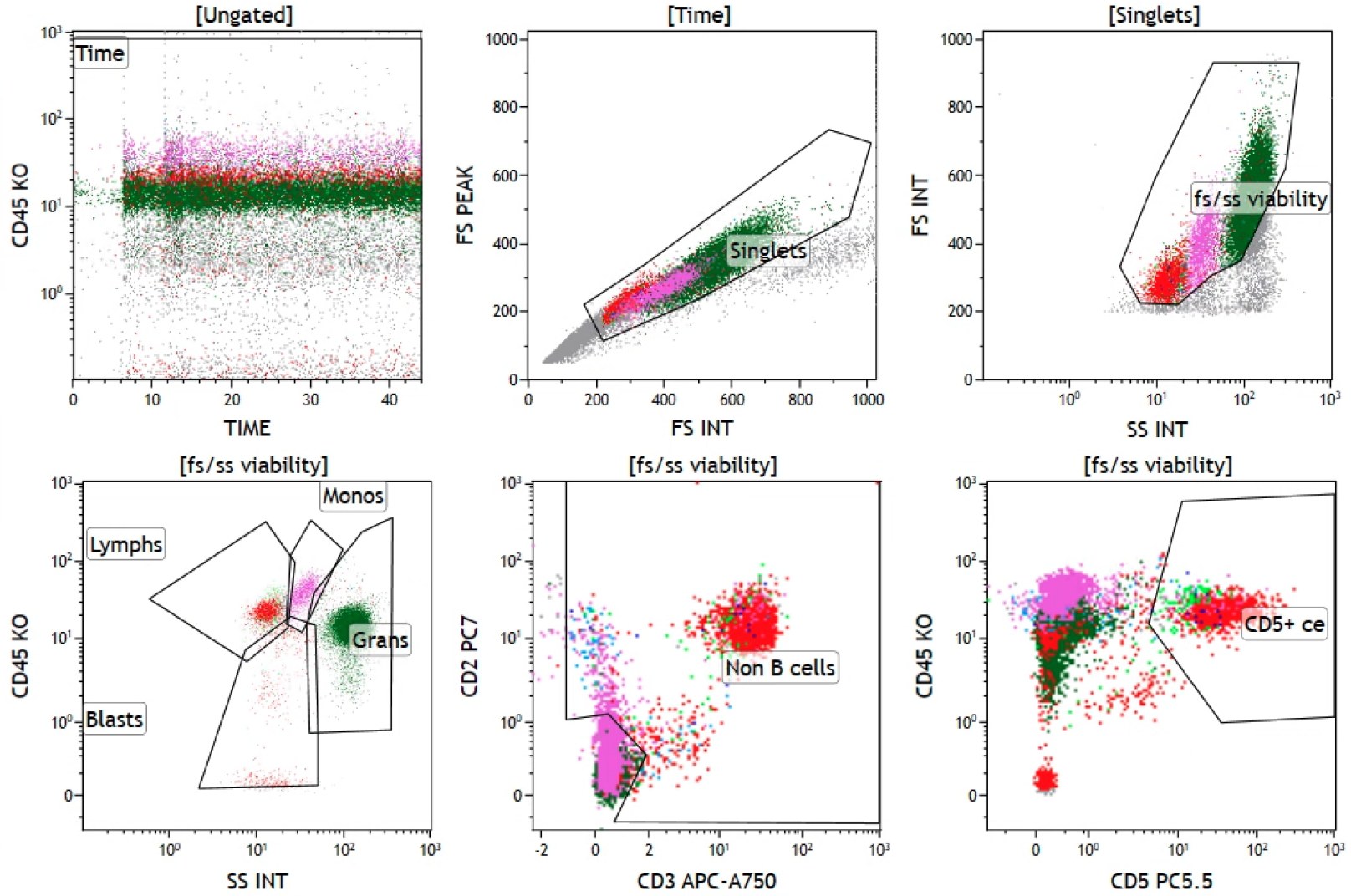
T cell aberrancies (vs CD3)



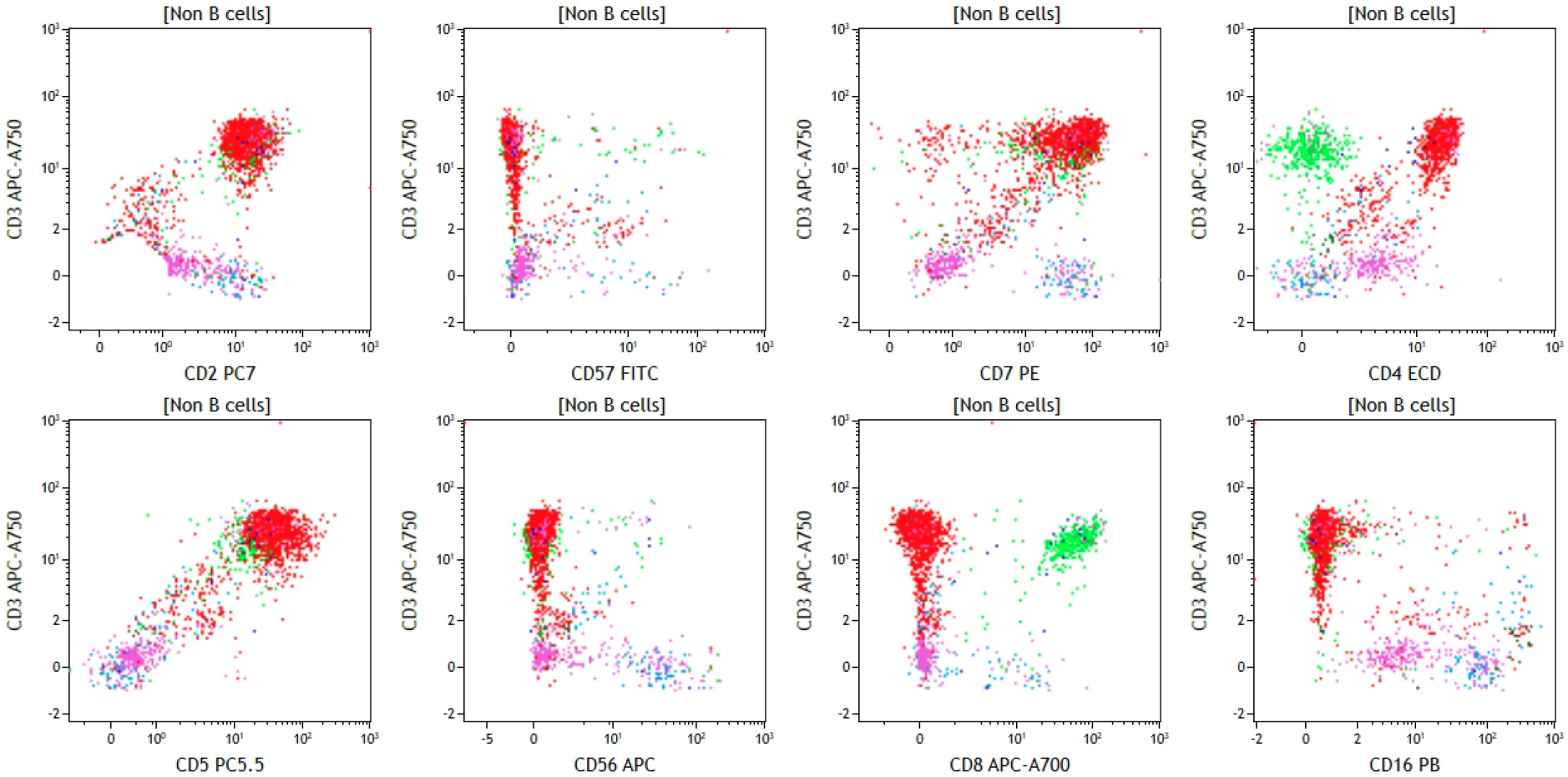
T cell aberrancies (vs CD2)



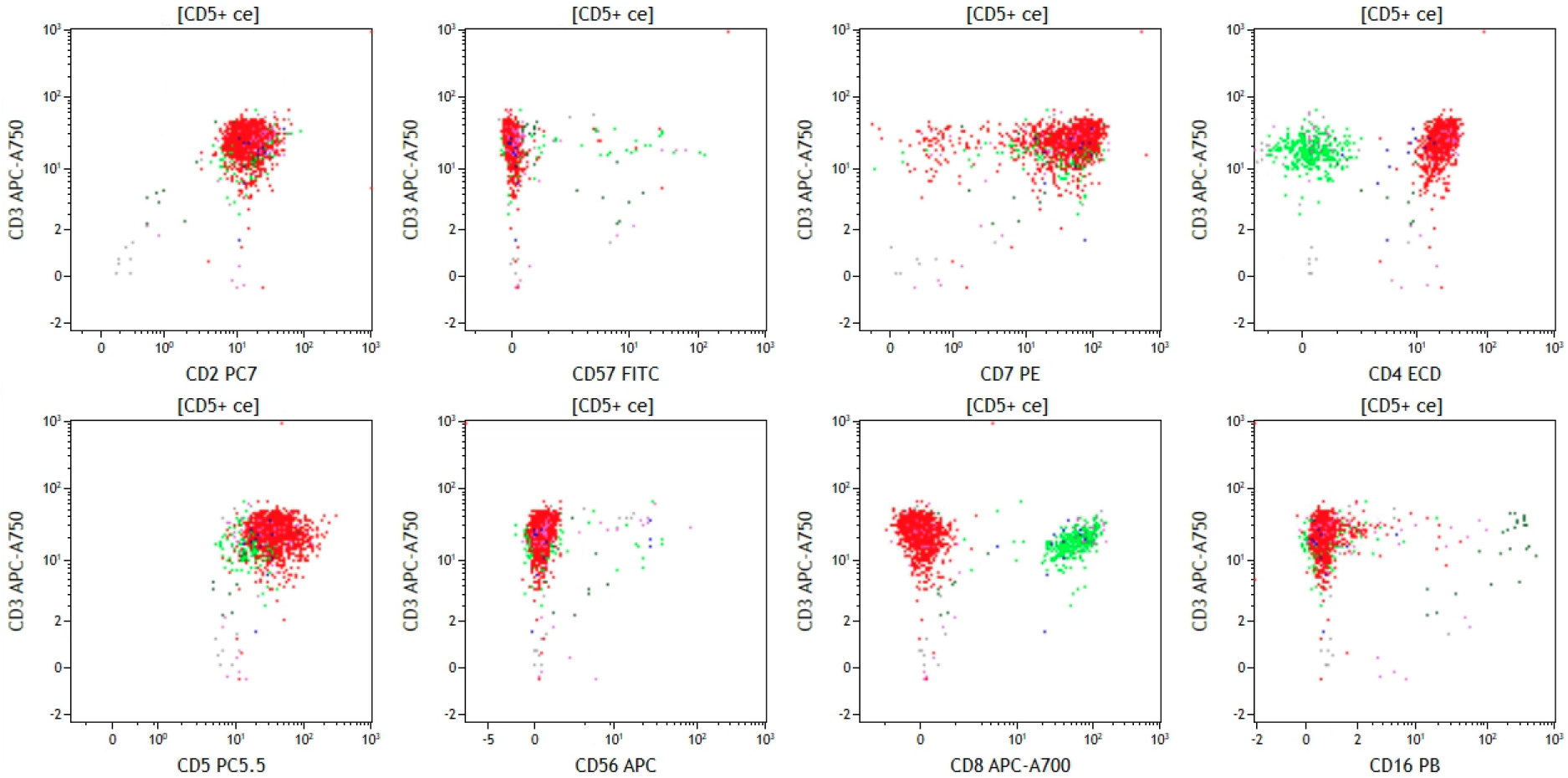
Other Gating Strategies



T cell aberrancies (vs CD3) on all non-B cells (CD3+ or CD2+ cells)



T cell aberrancies based on CD5+ events



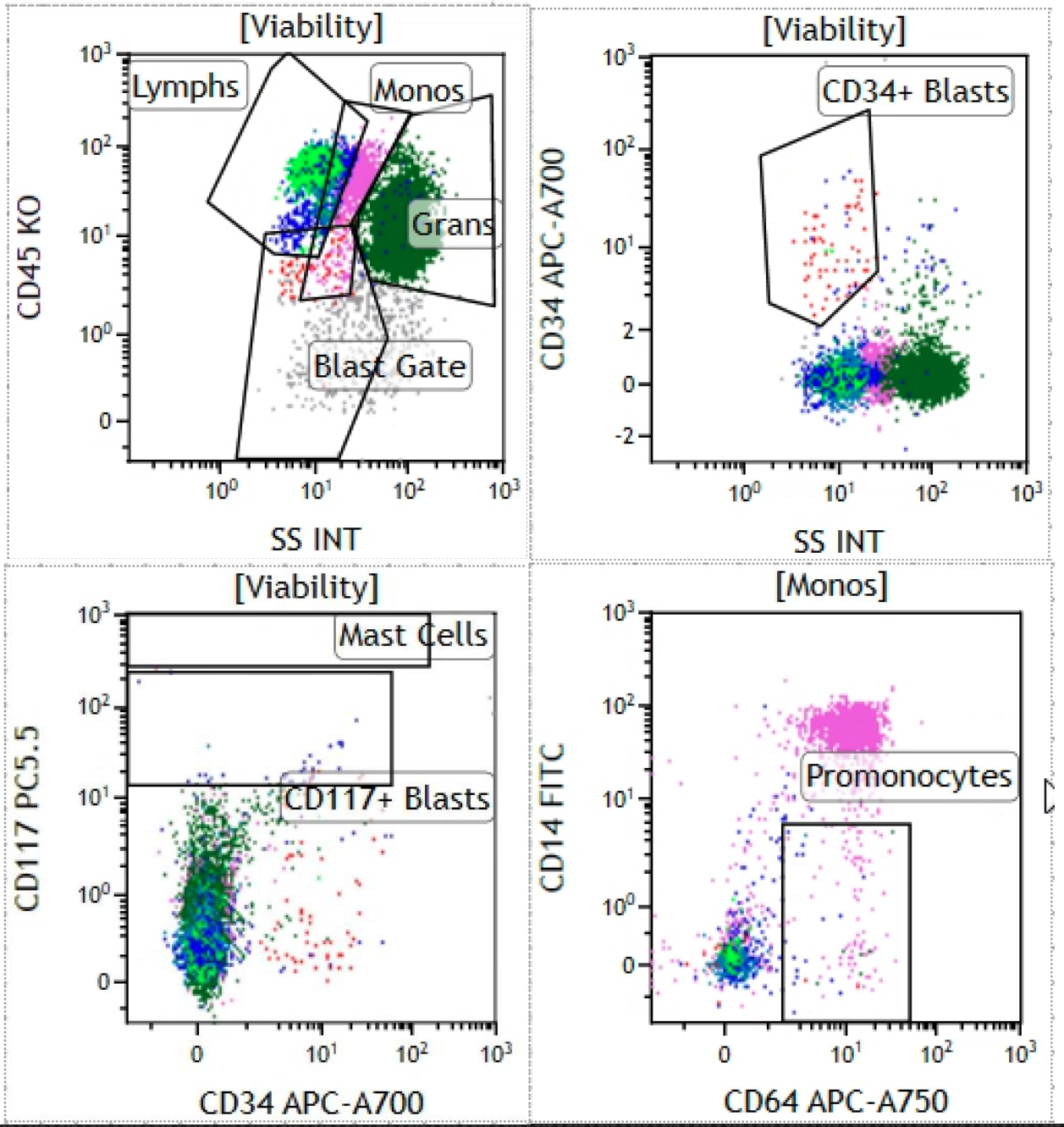
Gating Strategy for myelomonocytic leukemias

- Blasts and Blast Equivalents
- Myeloid Blasts – CD34+ without monocytic markers
- Promonocytes – CD34-/CD14-/CD64+
- Mature monocytes CD14+/CD64+

- Monoblast? – CD34+/- and or CD117+/- and CD64+

Gating

1. Drop Down Monocyte Gate
2. Look at blasts on CD34 and CD117
3. Look at expanded monocyte gate with CD64 and CD14



Summary

- Multiple avenues to a population
- Phenotypic aberrancies can render one strategy useless
- Backgating is your friend in a high dimensional platform
- Chose an input gate that maximizes information but minimizes ‘noise’
- Keep in mind what you gated on when looking at plots (where do these populations come from)

ARUP® LABORATORIES | HEALTH UNIVERSITY OF UTAH

ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.