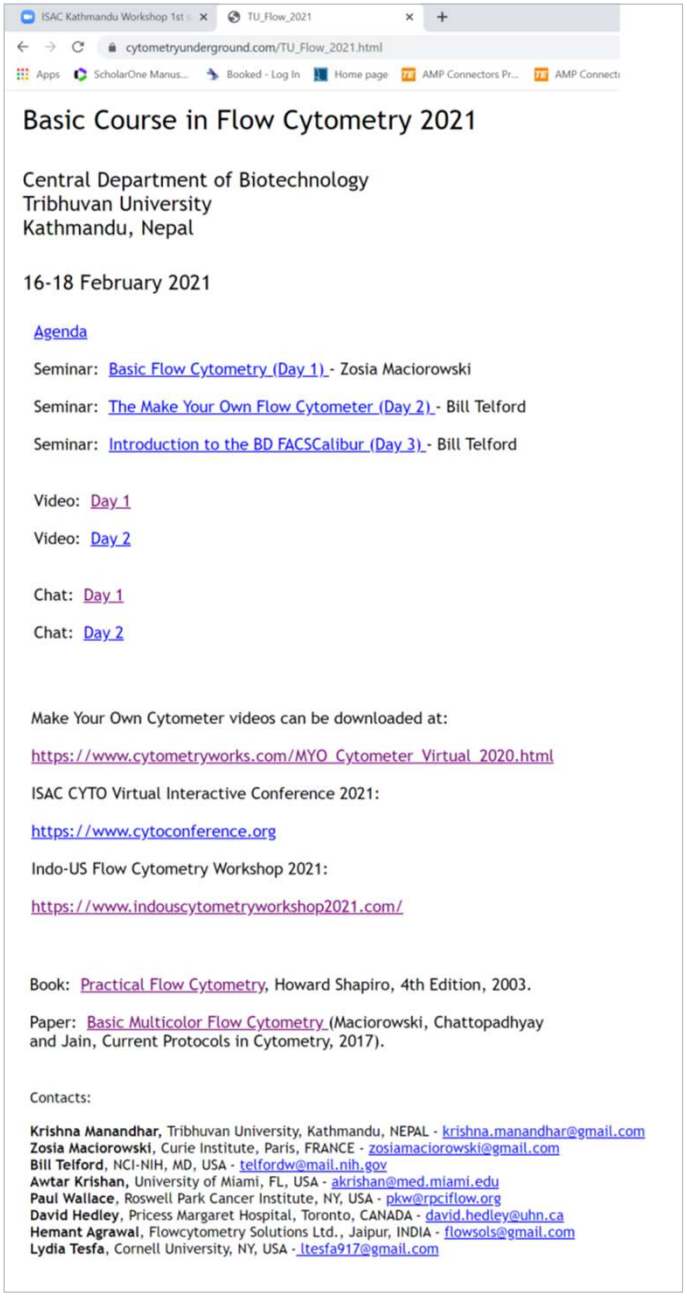

ISAC Kathmandu Workshop 1st | TU_Flow_2021

cytometryunderground.com/TU_Flow_2021.html

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Basic Course in Flow Cytometry 2021

Central Department of Biotechnology
Tribhuvan University
Kathmandu, Nepal

16-18 February 2021

Agenda

Seminar: Basic Flow Cytometry (Day 1) - Zosia Maciorowski

Seminar: The Make Your Own Flow Cytometer (Day 2) - Bill Telford

Seminar: Introduction to the BD FACSCalibur (Day 3) - Bill Telford

Video: Day 1

Video: Day 2

Chat: Day 1

Chat: Day 2

Make Your Own Cytometer videos can be downloaded at:

https://www.cytometryworks.com/MYO_Cytometer_Virtual_2020.html

ISAC CYTO Virtual Interactive Conference 2021:

https://www.cytoconference.org

Indo-US Flow Cytometry Workshop 2021:

https://www.indouscytometryworkshop2021.com/

Book: Practical Flow Cytometry, Howard Shapiro, 4th Edition, 2003.

Paper: Basic Multicolor Flow Cytometry (Maciorowski, Chattopadhyay and Jain, Current Protocols in Cytometry, 2017).

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David Hedley, Pricess Margaret Hospital, Toronto, CANADA - david.hedley@uhn.ca
Hemant Agrawal, Flowcytometry Solutions Ltd., Jaipur, INDIA - flowsols@gmail.com
Lydia Tesfa, Cornell University, NY, USA - ltesfa917@gmail.com

Workshop link:

https://www.cytometryunderground.com/TU_Flow_2021.html

CYTO Virtual Interactive Conference 2021:

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Indo-US Virtual Workshop 2021:

https://www.indouscytometryworkshop2021.com

Make Your Own Cytometer videos:

http://www.cytometryworks.com/MYO_Cytometer_Virtual_2020.html

1st Nepal Virtual Flow Cytometry Workshop

16-18 February 2021
Central Department of Biotechnology, Tribhuvan University
Kathmandu Nepal

Day 3 **18 February 2021**

17:00 – 18:30 **Operation of the BD FACSCalibur Flow Cytometer** (Interactive)

Bill Telford and multiple faculty

Live demonstration of the features and operation of a BD FACSCalibur flow cytometry, including simple cell analysis. With live commentary by multiple faculty.

18:30 – 19:00 Discussion

All times are Nepal Time.

All meetings start at 17:00 Kathmandu time (6:15 Eastern USA time, 12:15 Paris time).

https://www.cytometryunderground.com/TU_Flow_2021.html

BD Biosciences FACSCalibur

Introducing FACSCalibur.

A New Legend Begins.

For over two decades, Becton Dickinson's tradition of advanced technology and innovative solutions has been legendary in the industry. Now with FACSCalibur,™ our new automated, four-color flow cytometry system, the legend continues.

FACSCalibur is the only four-color benchtop system that both analyzes and sorts, making it the ideal choice for labs performing multiple applications. With its automated loader, broad range of reagents, and powerful software applications, FACSCalibur offers the fast throughput necessary to meet productivity requirements and provides the flexibility and performance that are essential for today's laboratory environment.

FACSCalibur can quickly perform routine tests for immunophenotyping, lymphocyte subset analysis with absolute counts*, reticulocyte enumeration, and DNA analysis*. It can give investigators the sensitivity and resolution so crucial for sophisticated cellular work in genetics, molecular biology, multicolor analysis, and immune function. And with FACSCalibur, you can sort cells of interest for further study, adding another dimension to the information you gain.

To discover how the new FACSCalibur system can make a difference in the productivity and performance of your laboratory, contact your local Becton Dickinson representative today.

FACSCalibur: A new legend begins.

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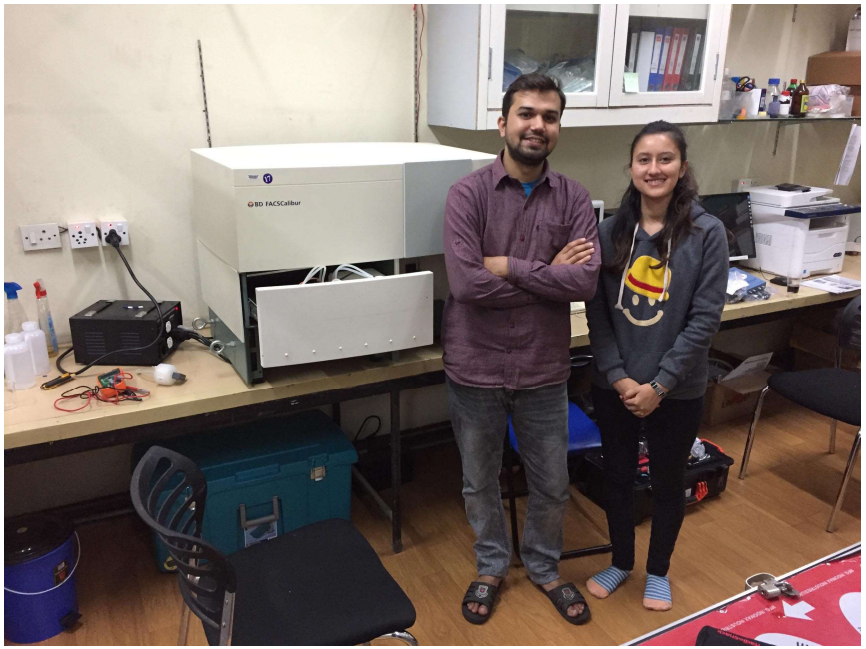
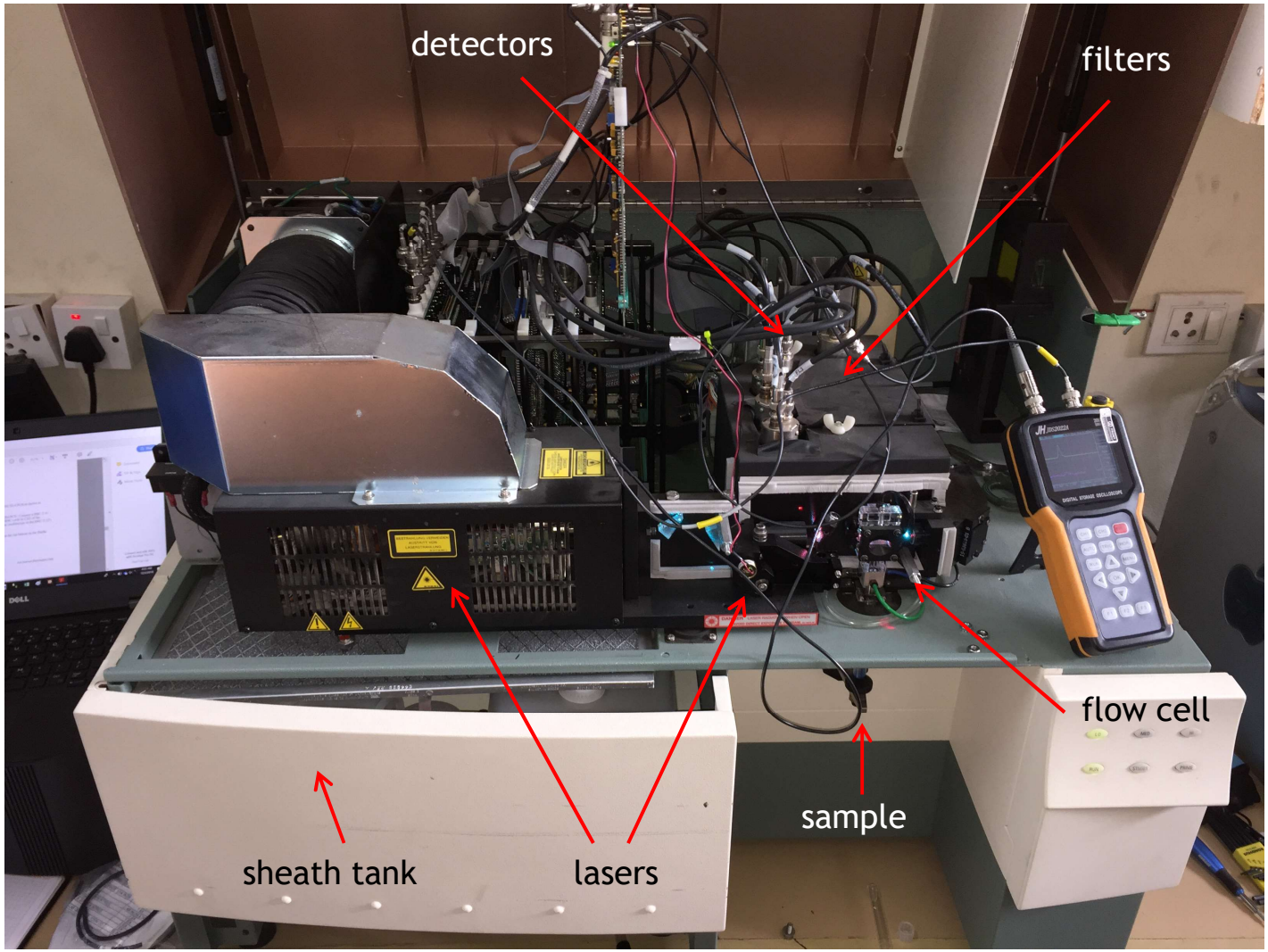
BD FACSCalibur at Tribhuvan University



BD FACSCalibur at Tribhuvan University

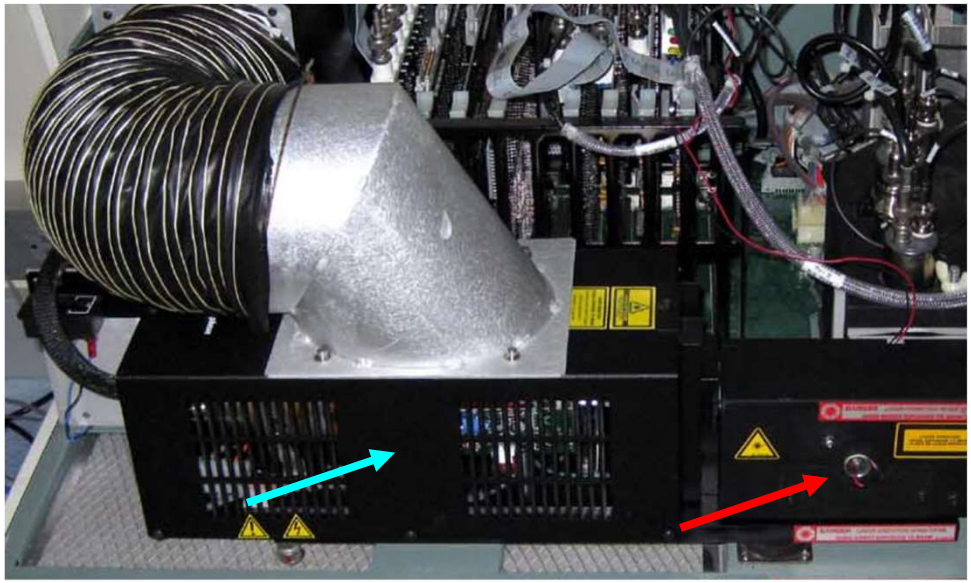
BD FACSCalibur flow cytometer

Two lasers, four colors



Lasers in flow cytometry

Like most cytometers, the FACSCalibur has a **blue-green 488 nm laser**. This wavelength is useful for light scatter analysis, and can excite many fluorescent probes.



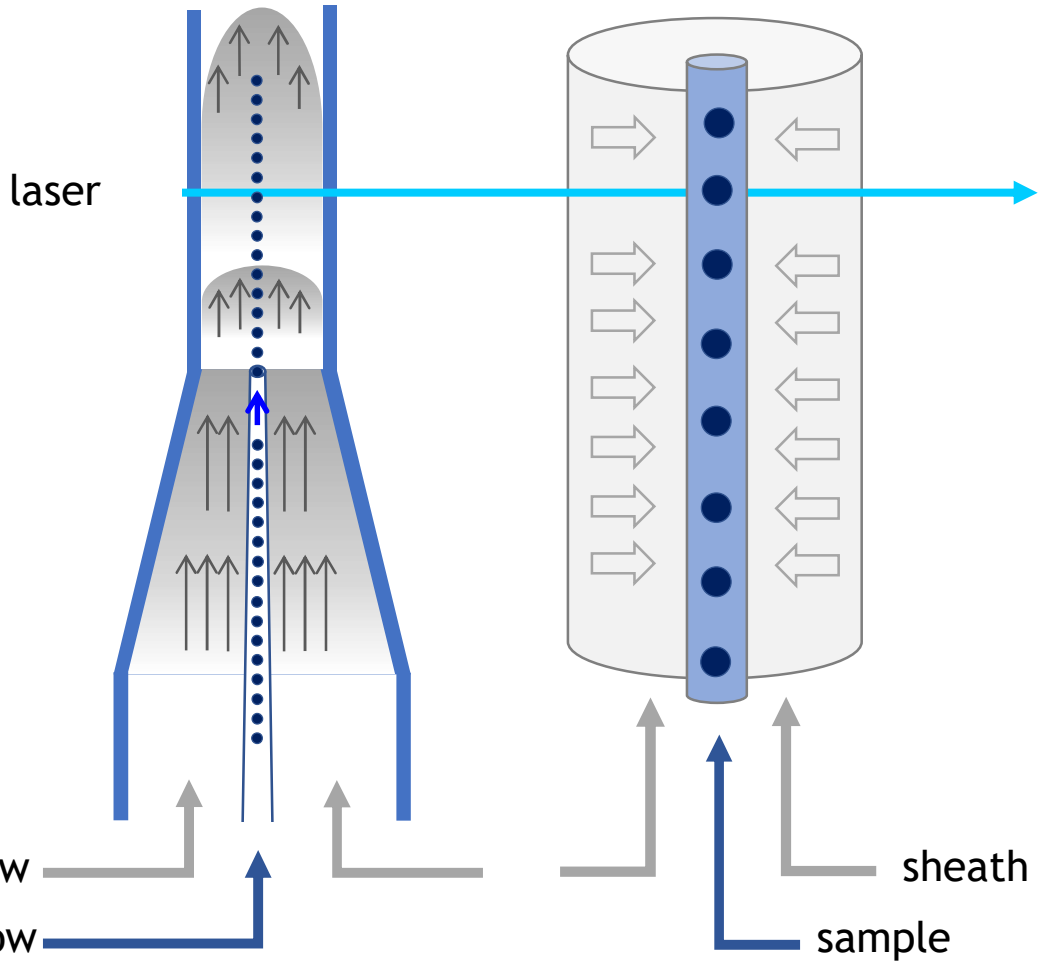
It also has a red 640 nm laser, allowing excitation of more probes. Most cytometers now have more than one laser wavelength (some many more).

Delivering the cell sample to the laser beam

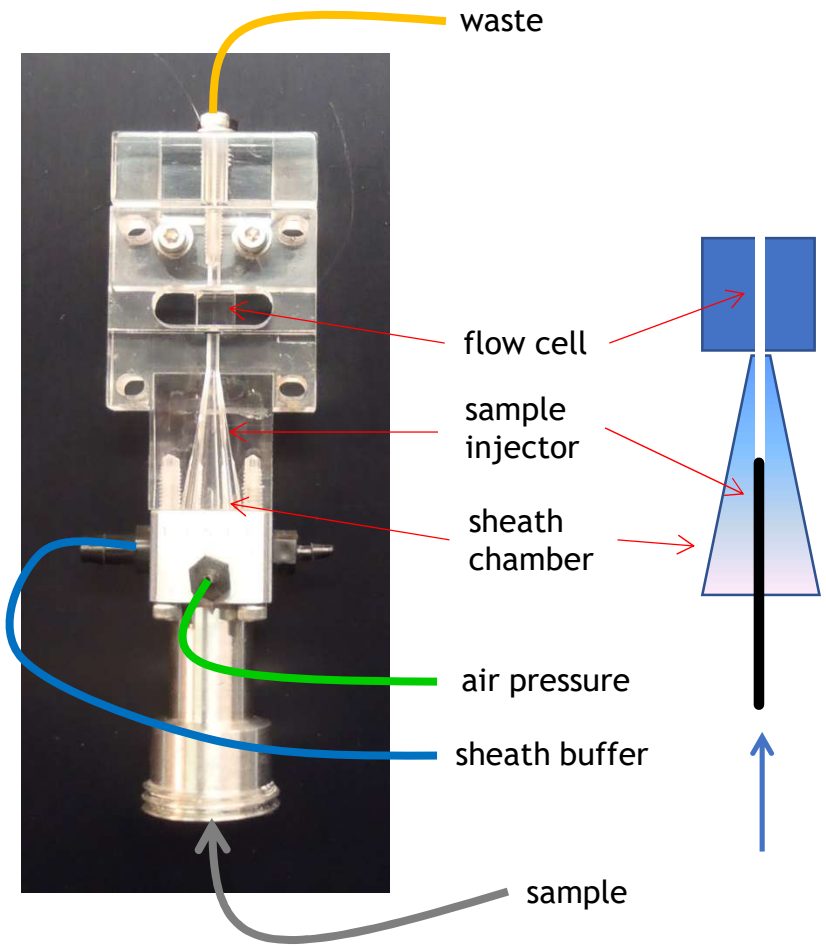
We inject our **cell sample** (in a liquid suspension) into an outer cylinder of moving buffer – the **sheath** stream.

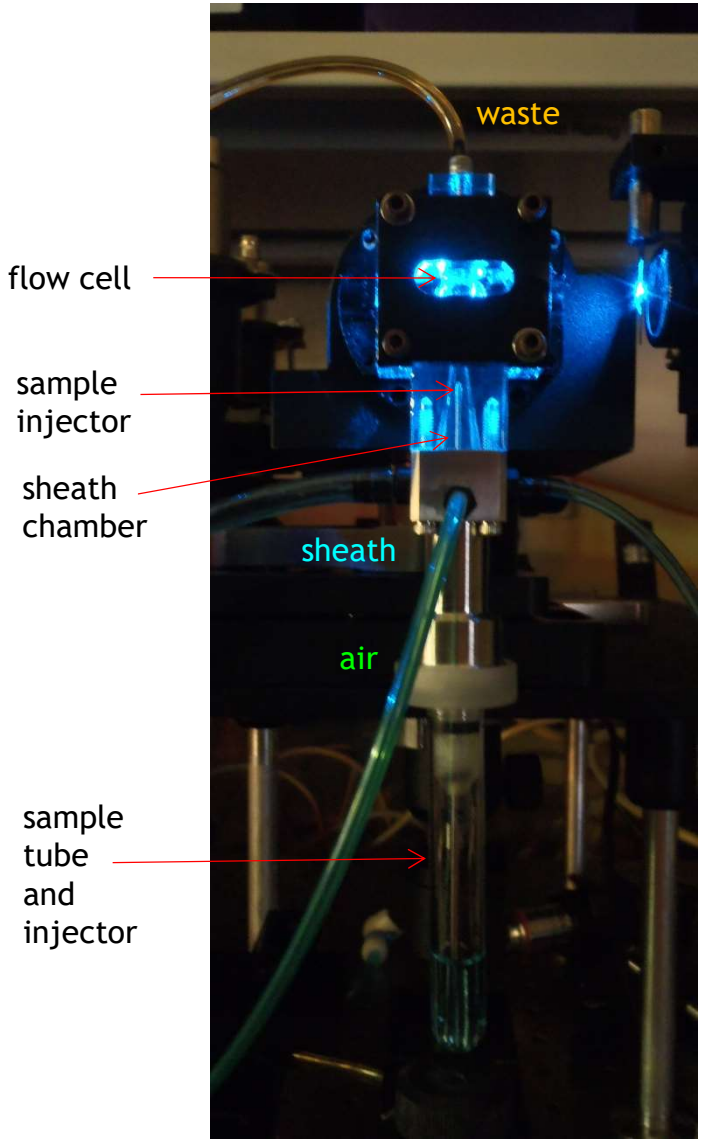
The sheath stream both pulls and "focuses" the inner sample stream into a very small area, allowing good illumination with a laser.

This is called **hydrodynamic focusing**, and is essential for sensitive analysis by flow cytometry.



BD FACSCalibur flow cell



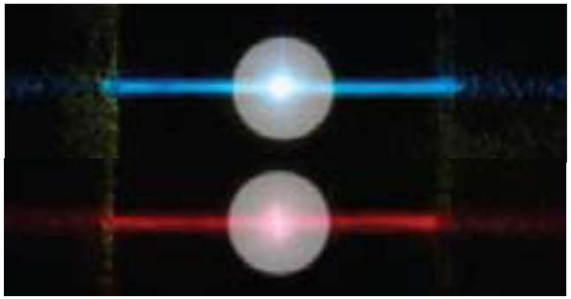


BD Biosciences FACSCalibur lasers

Blue-green 488 nm — fluorescein, PE, PerCP

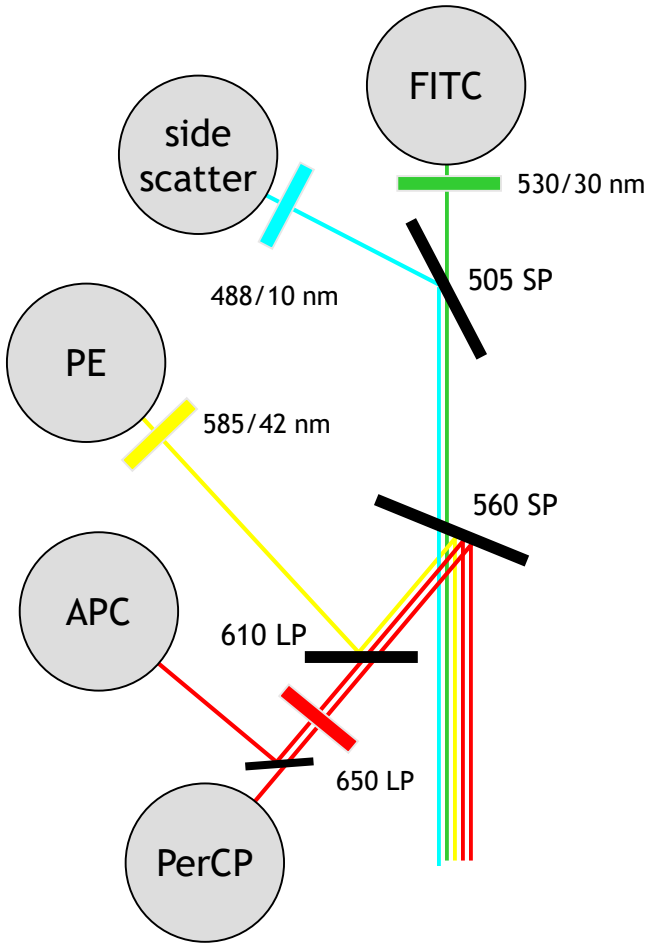
Red 620-640 nm — APC

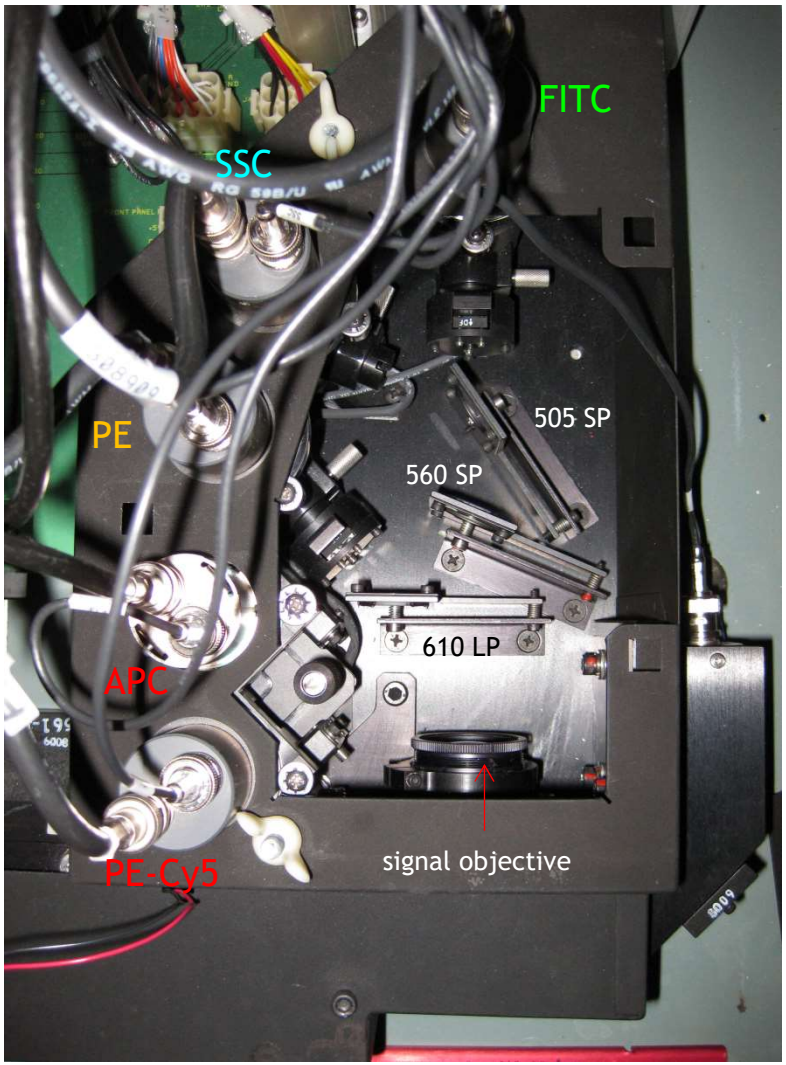
The FACSCalibur has two lasers. Three fluorescent probes can be detected using the blue-green 488 nm laser, and one with the red 640 nm laser.



BD Biosciences FACSCalibur detectors

Four fluorescence detectors, three off the 488 nm, one off the 640 nm laser.



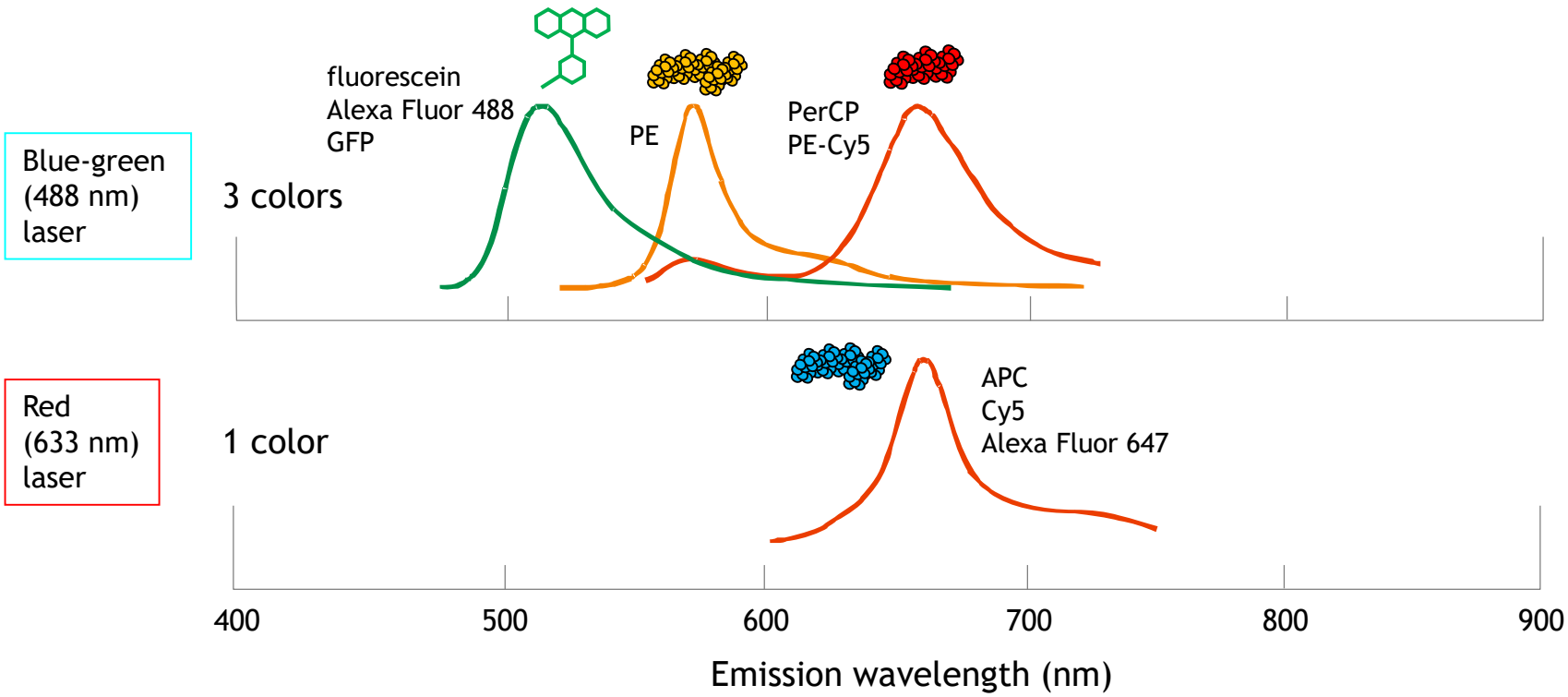


BD Biosciences FACSCalibur lasers and detectors

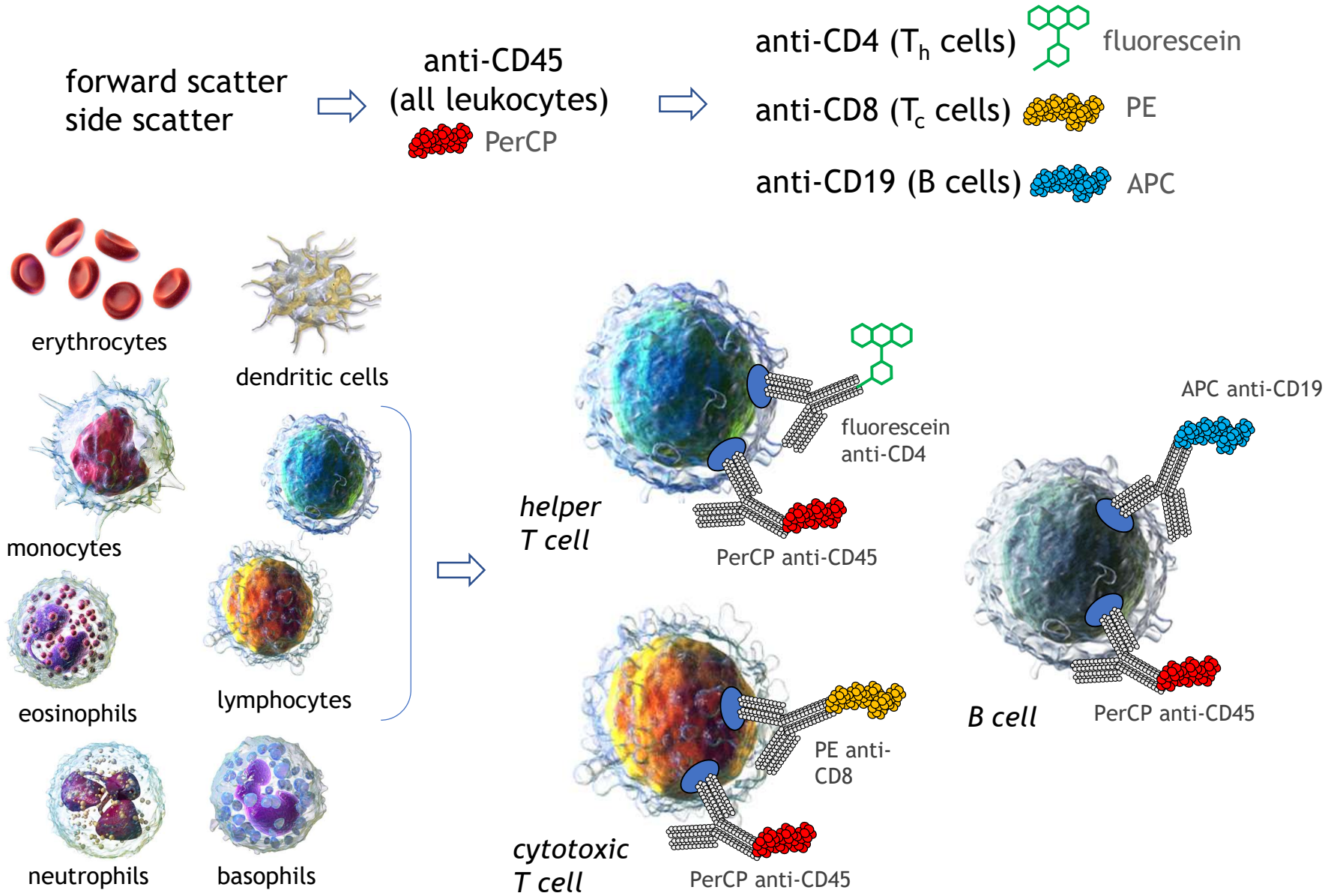
The FACSCalibur can detect up to four colors.

Fluorescein is the most common green fluorochrome, but Alexa Fluor 488 and GFP can be detected too. PerCP or PE-Cy5 can be detected in the long red detector.

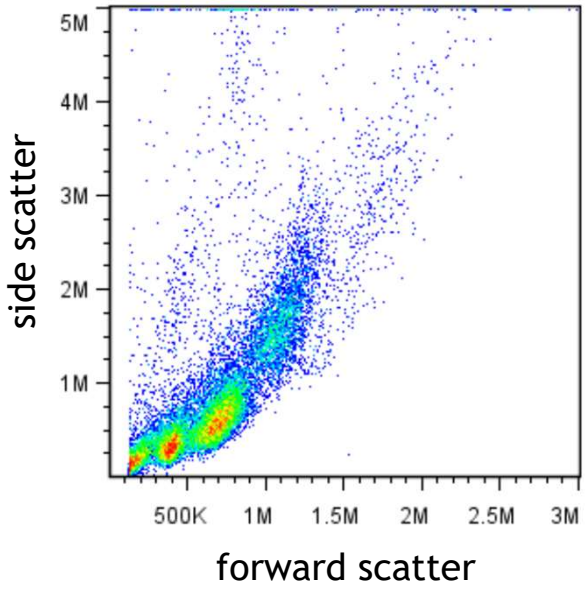
APC is detected using the red laser, but Cy5 and Alexa Fluor 647 are used too.

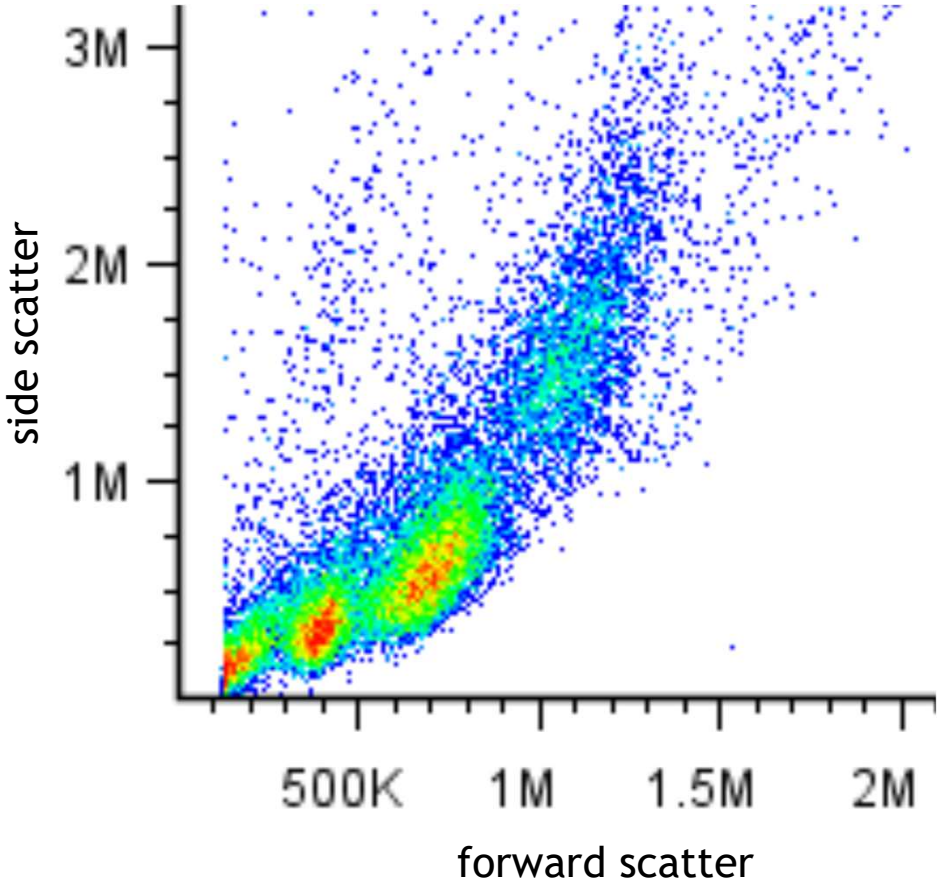


Four color analysis of peripheral blood

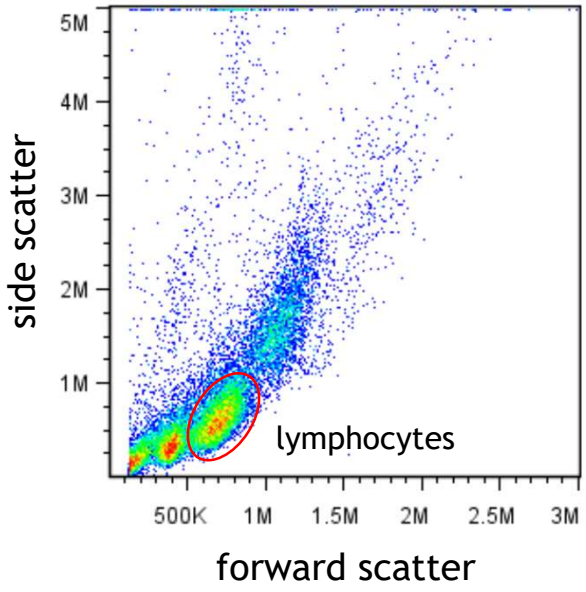


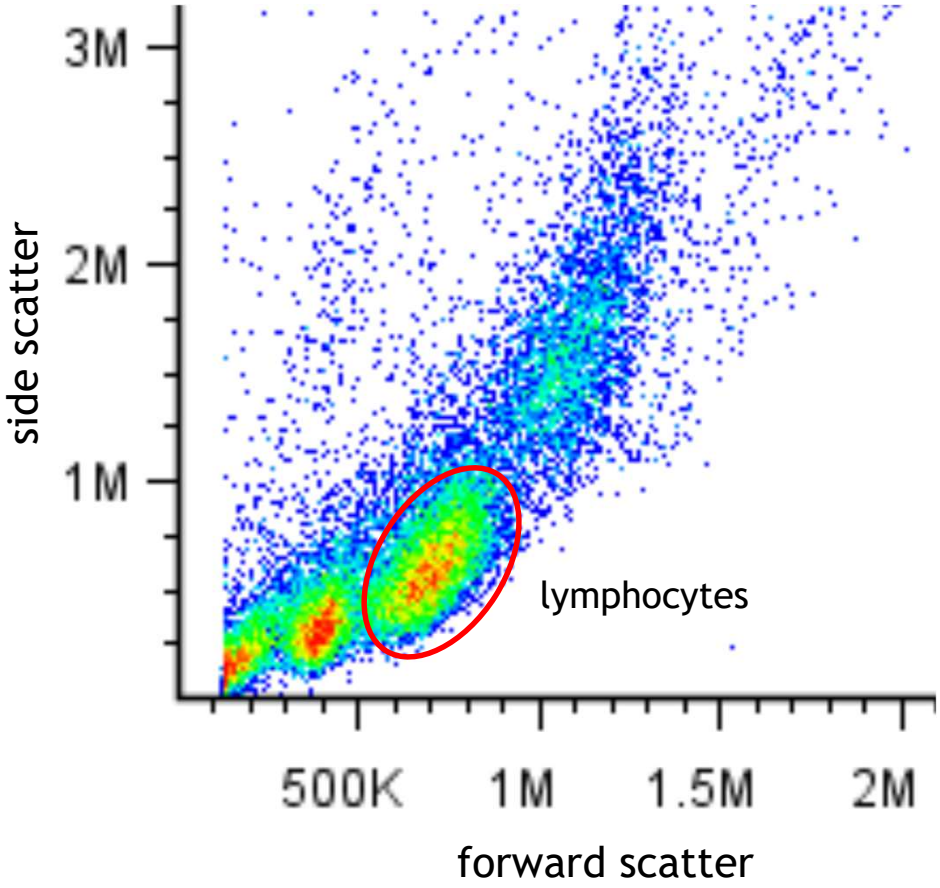
Labeling peripheral blood for flow cytometry





Labeling peripheral blood for flow cytometry





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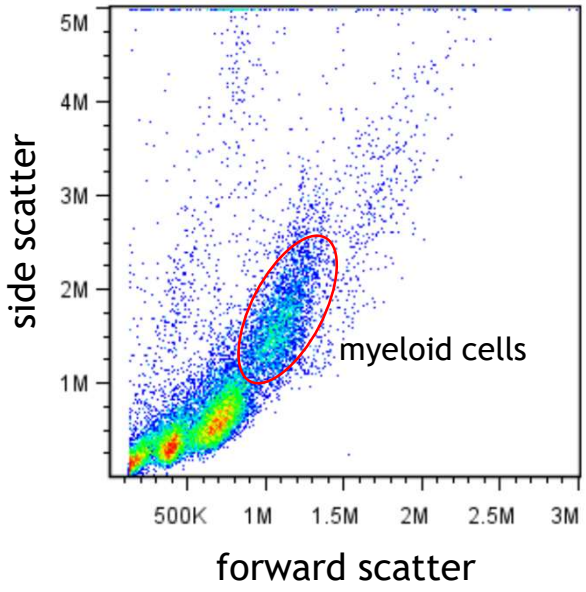

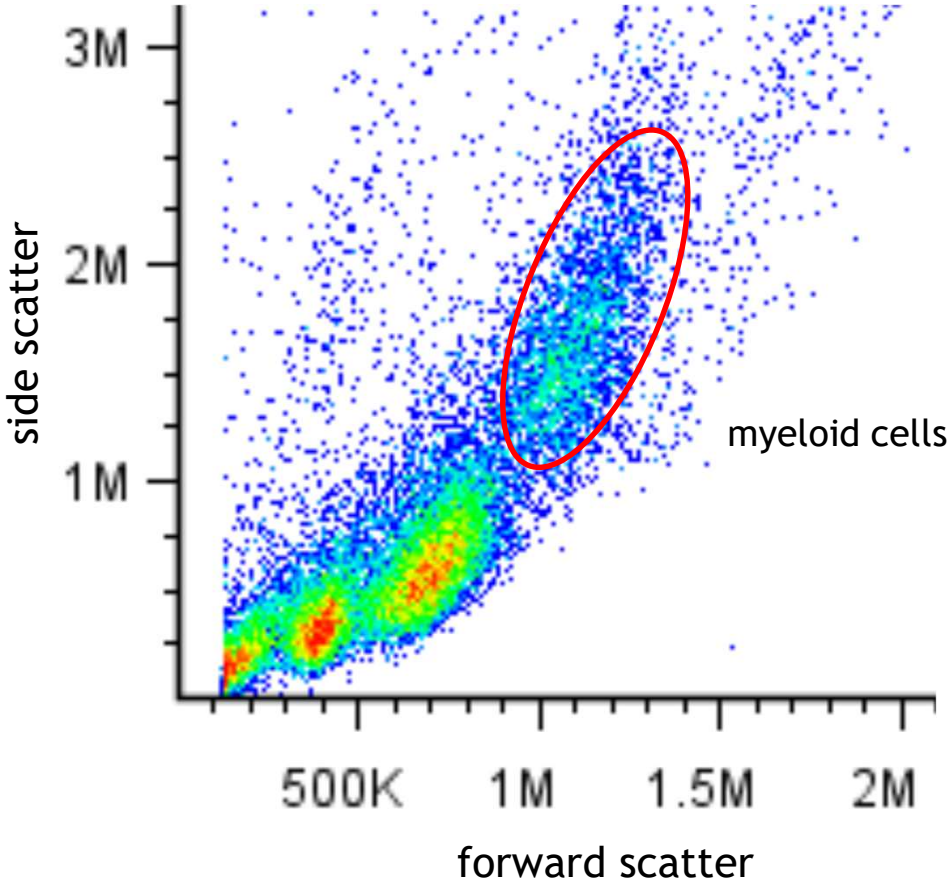

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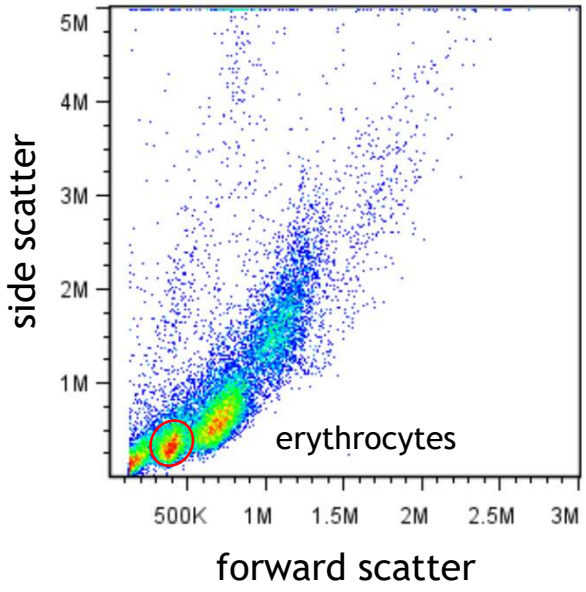

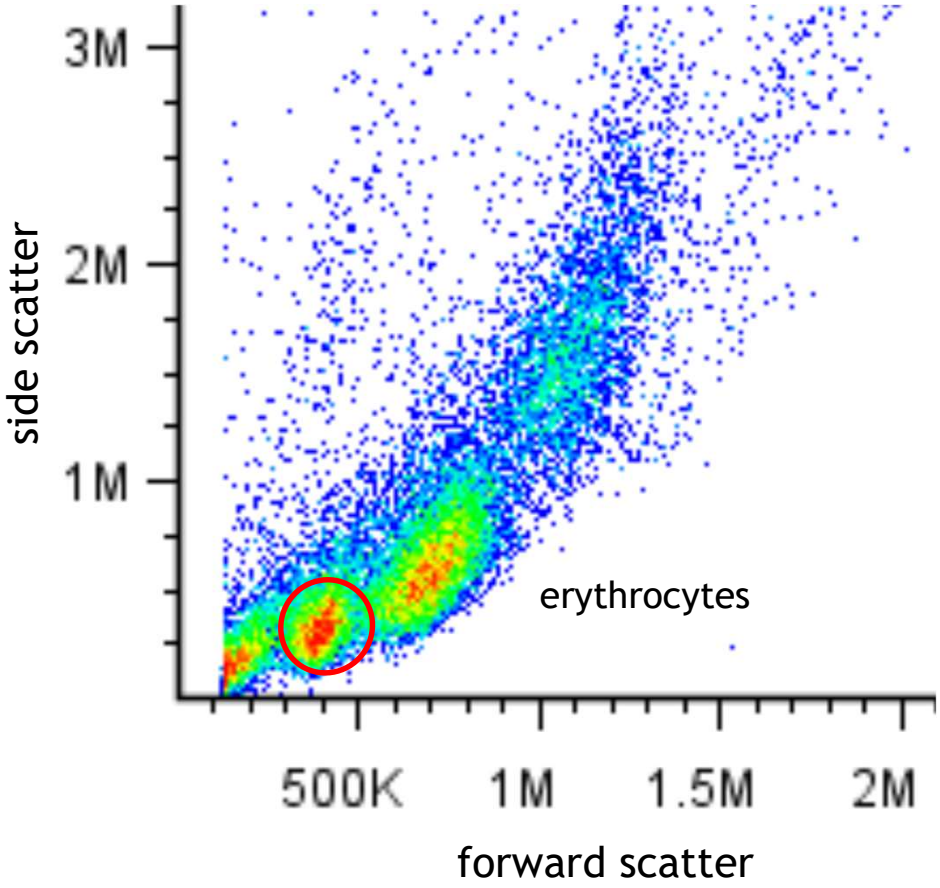

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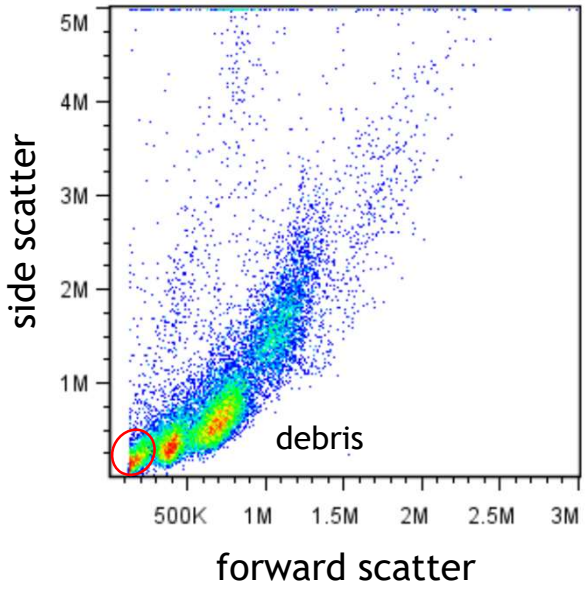
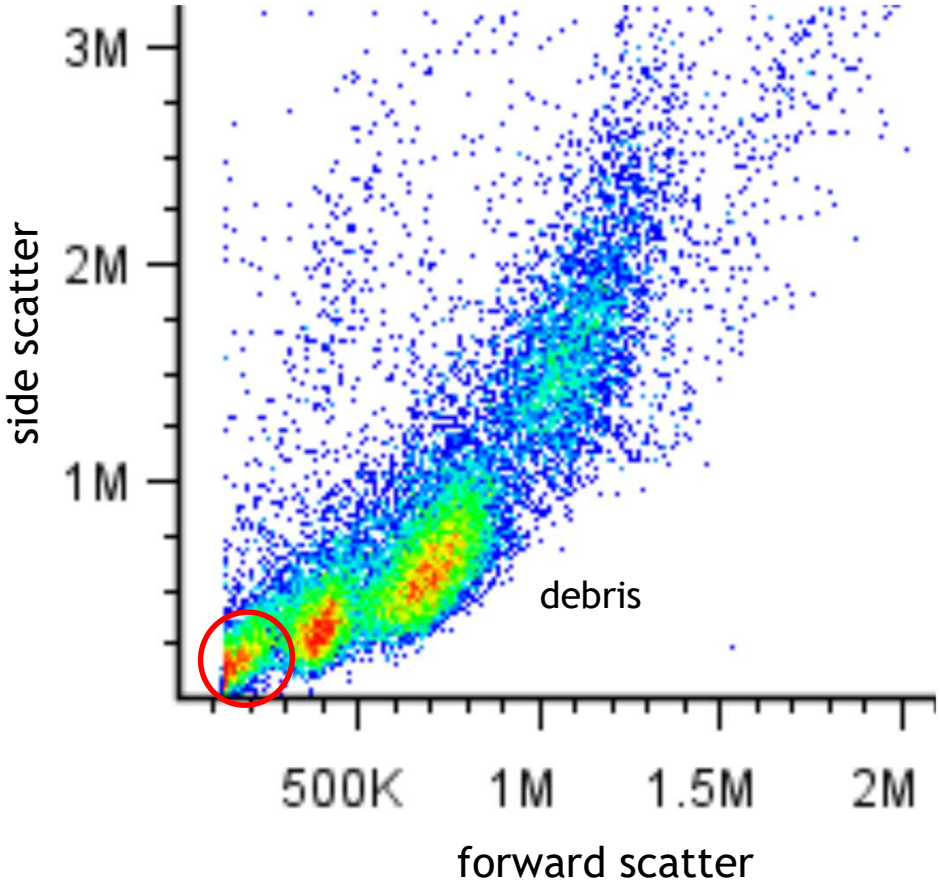
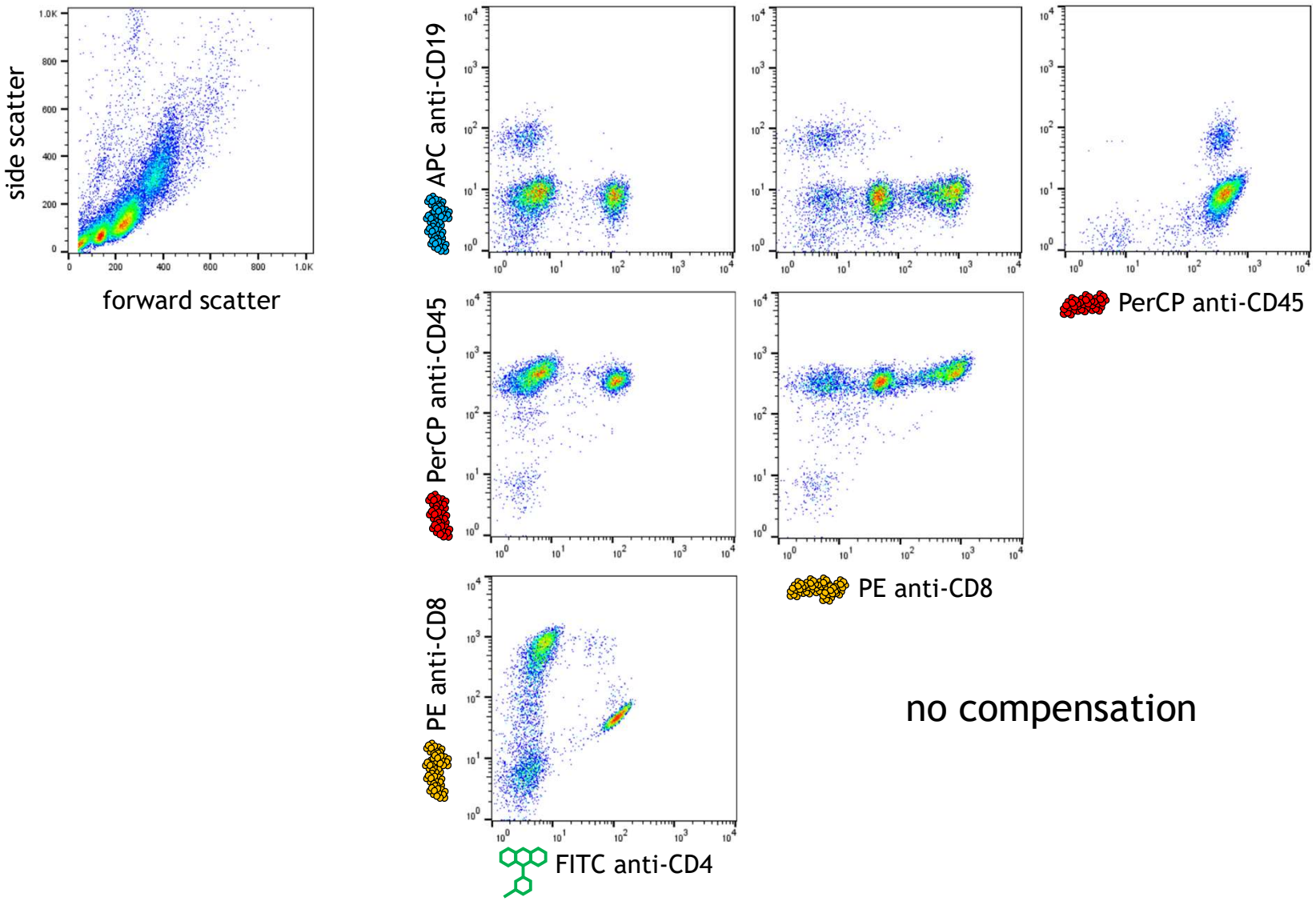


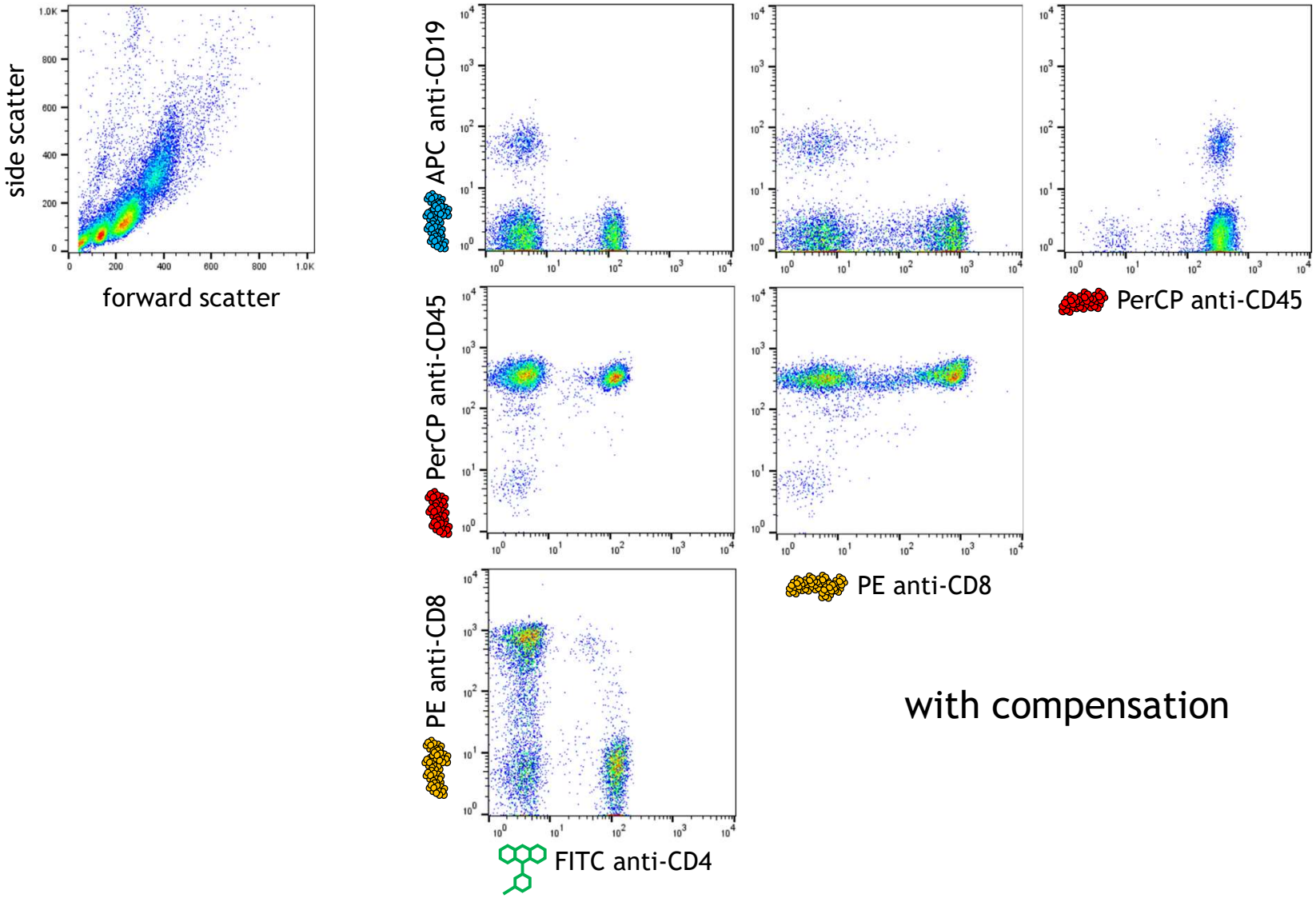


Labeling peripheral blood for flow cytometry



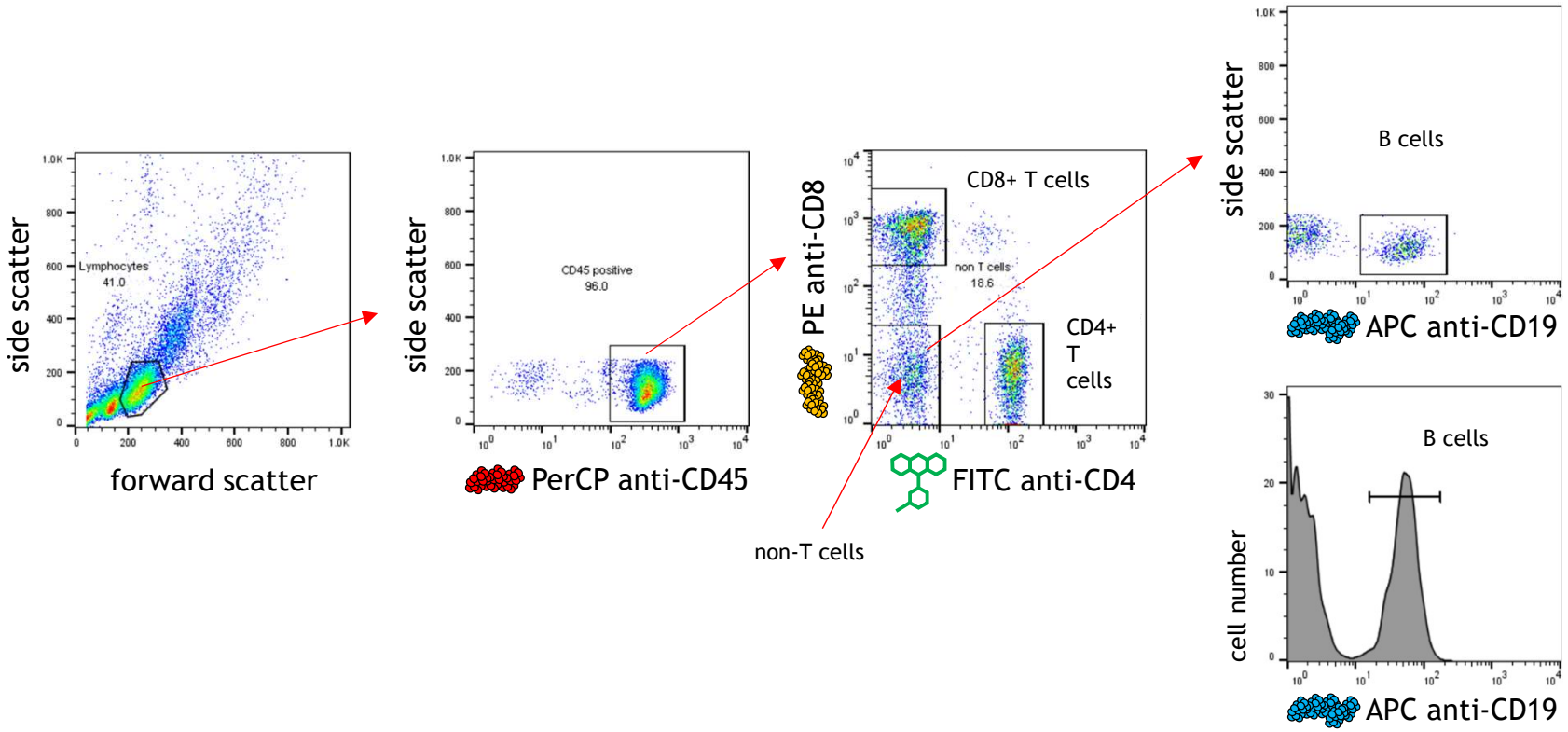
no compensation

Labeling peripheral blood for flow cytometry



with compensation

Labeling peripheral blood for flow cytometry



Compensation

Some of these fluorochromes overlap spectrally with others.

Some are excited by both laser wavelengths and have similar emission spectra.

We will need to determine the compensation matrix for these fluorochromes (either manually or automatically).