2023 금연 연구회 제7차 심포지엄

쉽게 배우는 유세포 분석기법과 담배추출물을 활용한 실험예시

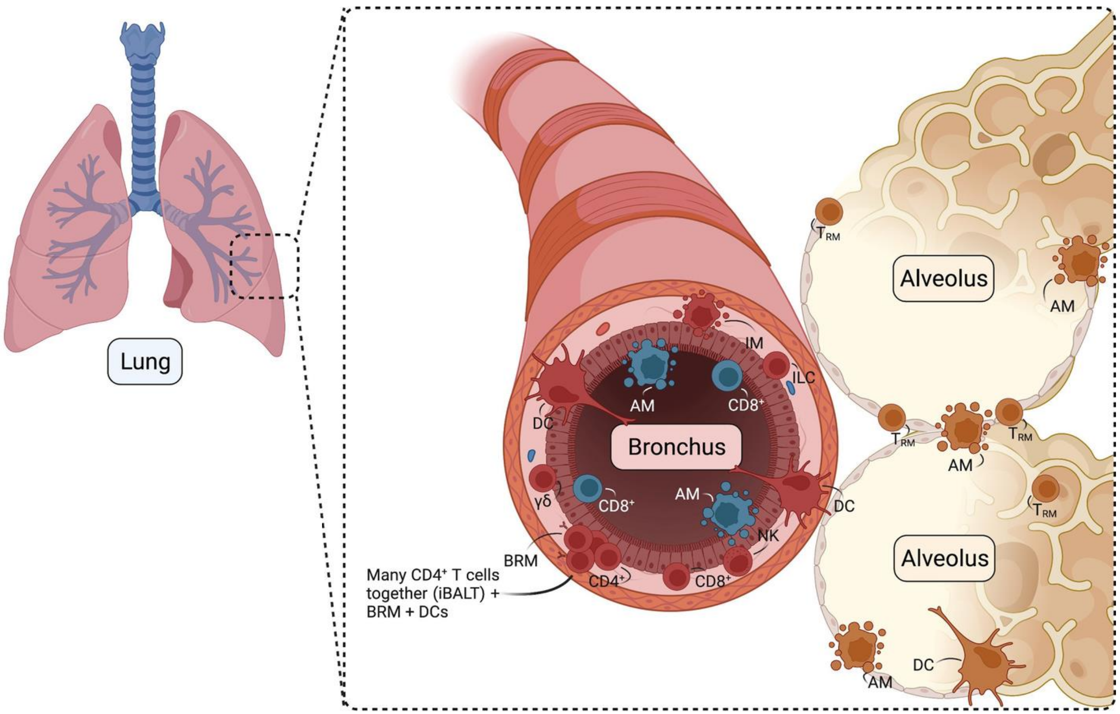
충남대학교병원 호흡기내과

김덕기

유세포 분석(flow cytometry)
: An essential tool for immunological studies

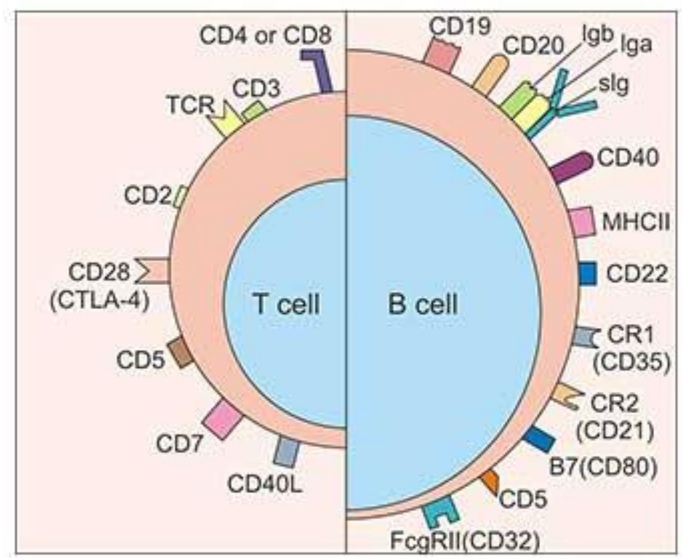
Flow cytometry

: The measurement (-metry) of cell (cyto-) characteristics and properties in motion (flow)



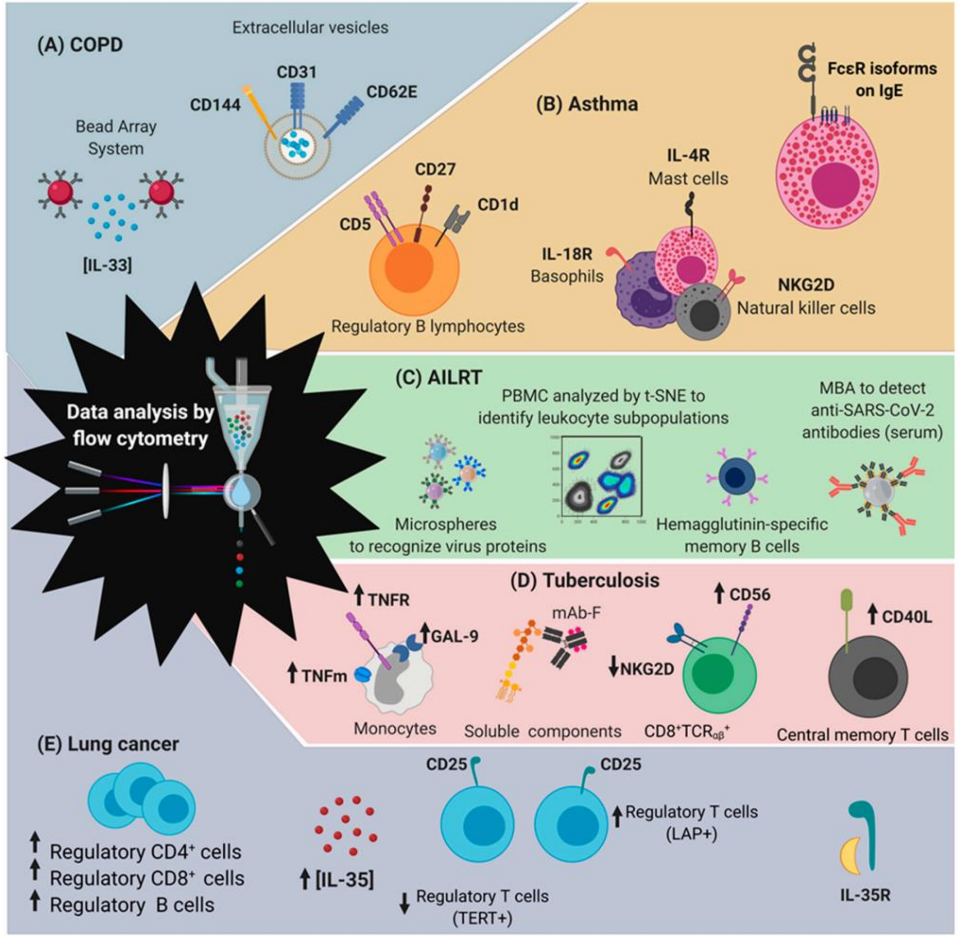
Zazara et al., 2022, Semin. Immunopathol.

Each type of immune cell differentially expresses marker proteins

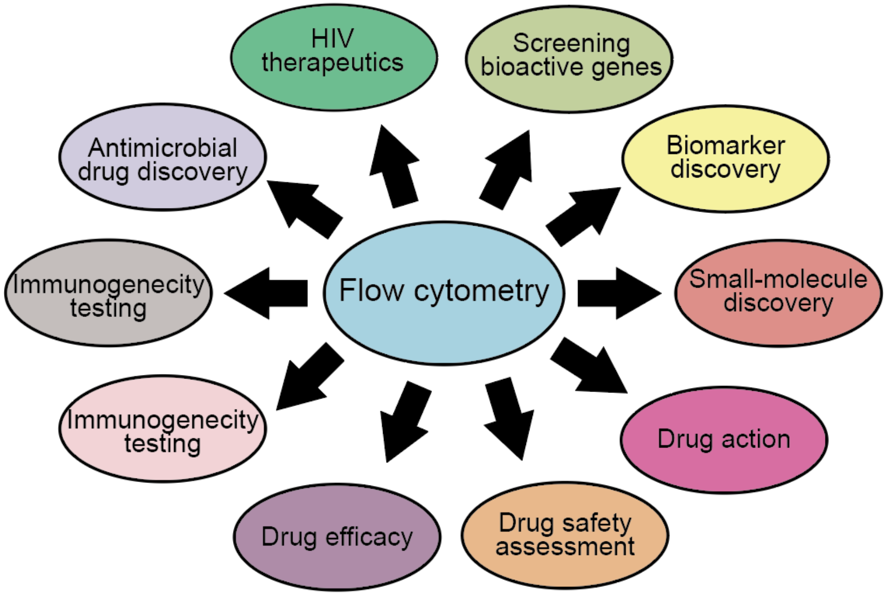


… allows for the identification and characterization of immune cell subsets within a heterogenous population…!

유세포 분석의 활용 범위

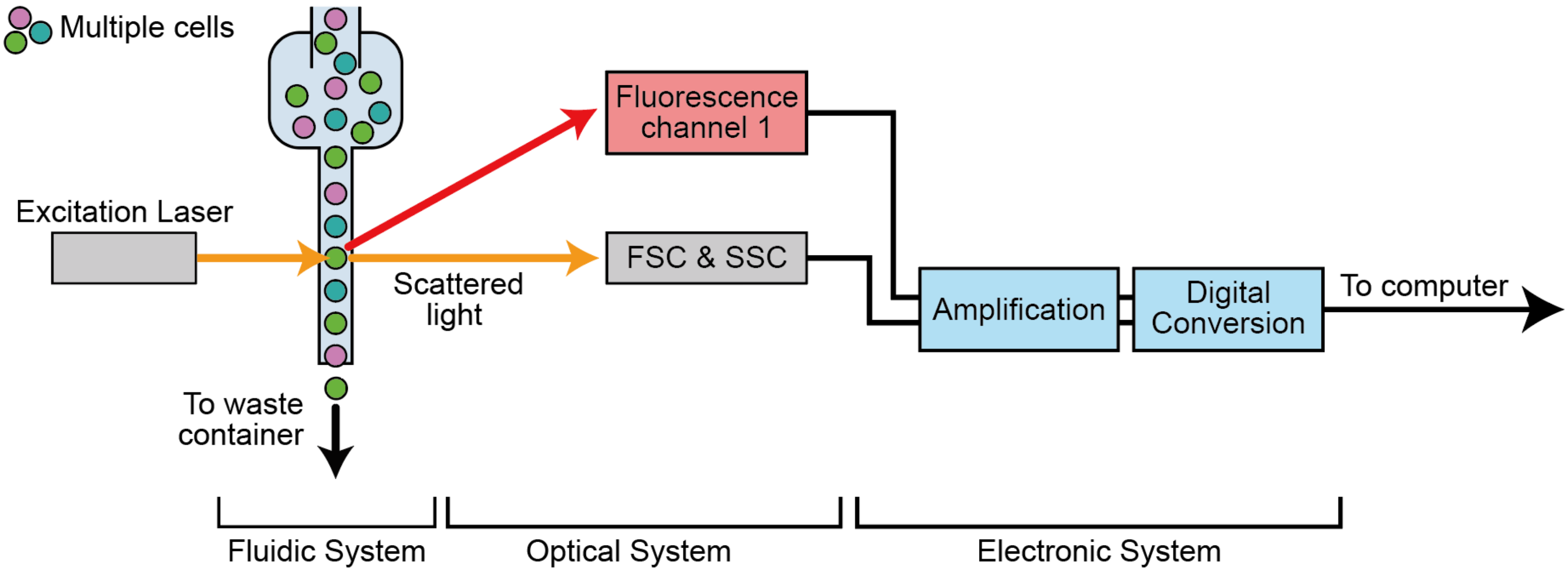


Julio et al., 2020, Int. J. Mol. Sci.

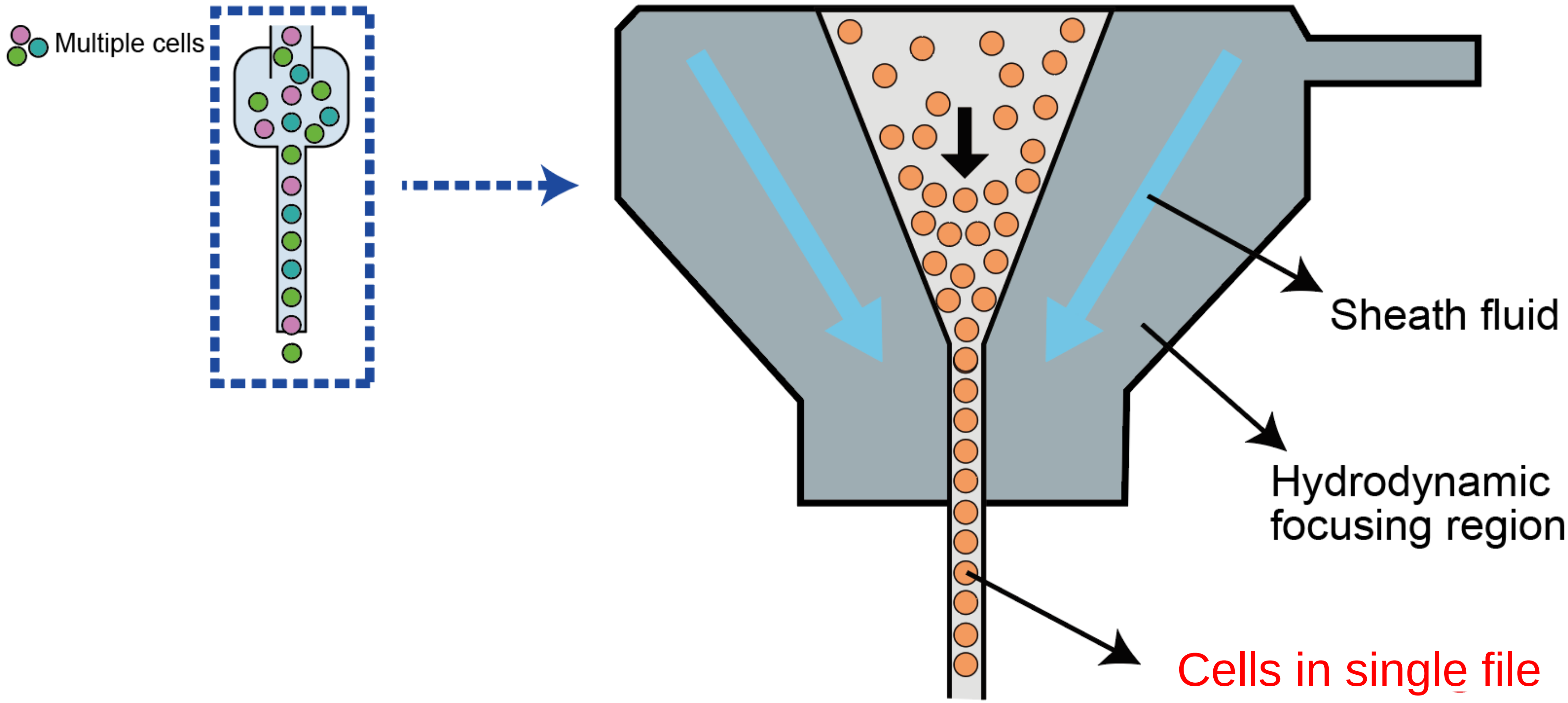


유세포 분석의 작동 원리

→ 유세포 분석은 단일 또는 다중 **레이저**를 사용하여 (형광이 표지된) **단일 세포**의 **다중 파라미터 분석**을 하는 기법입니다.

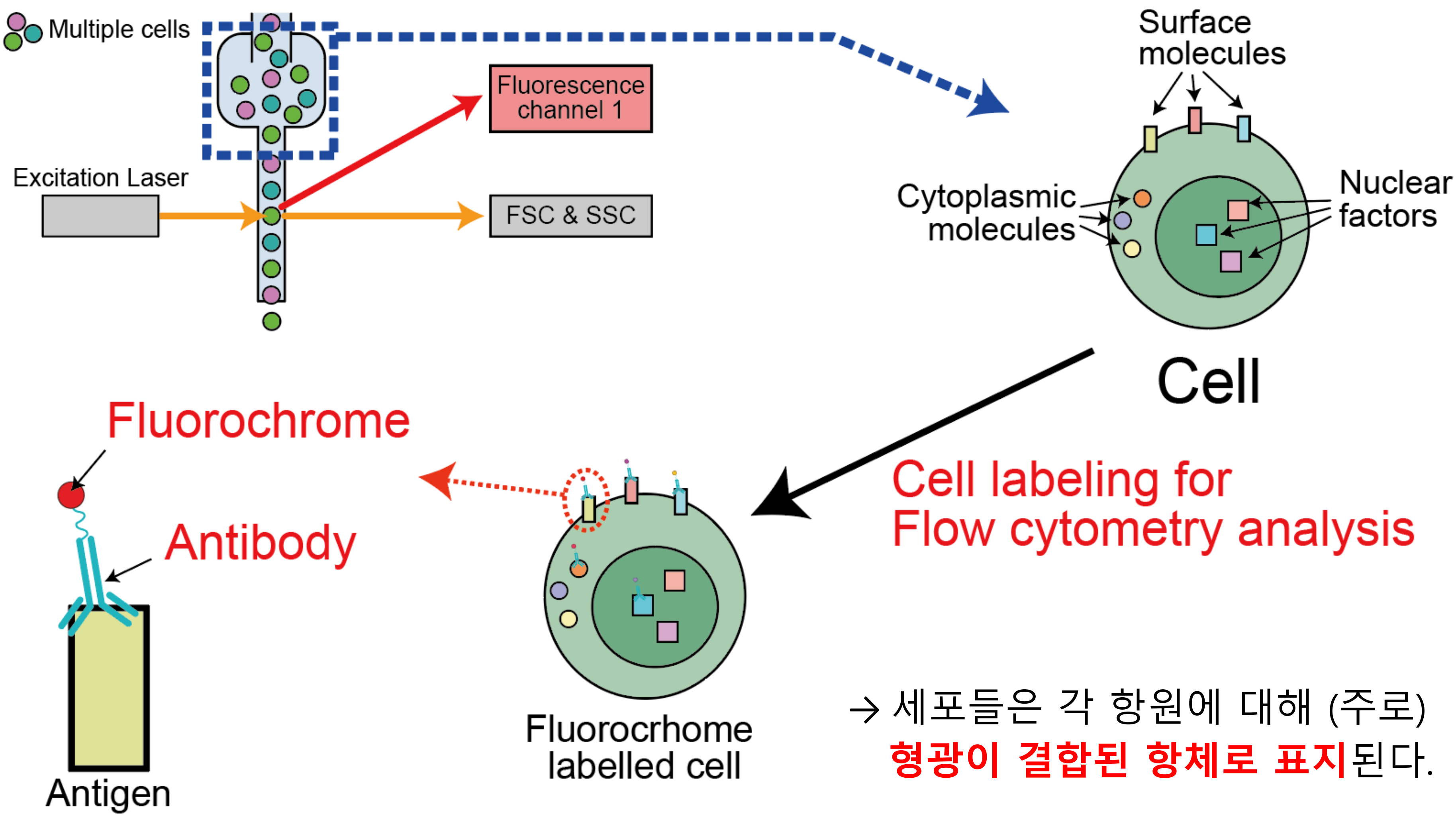


유세포 분석의 작동 원리 – Fluidic system



→ Fluidic system은 **세포 하나하나의 데이터**를 얻게 만들어준다.

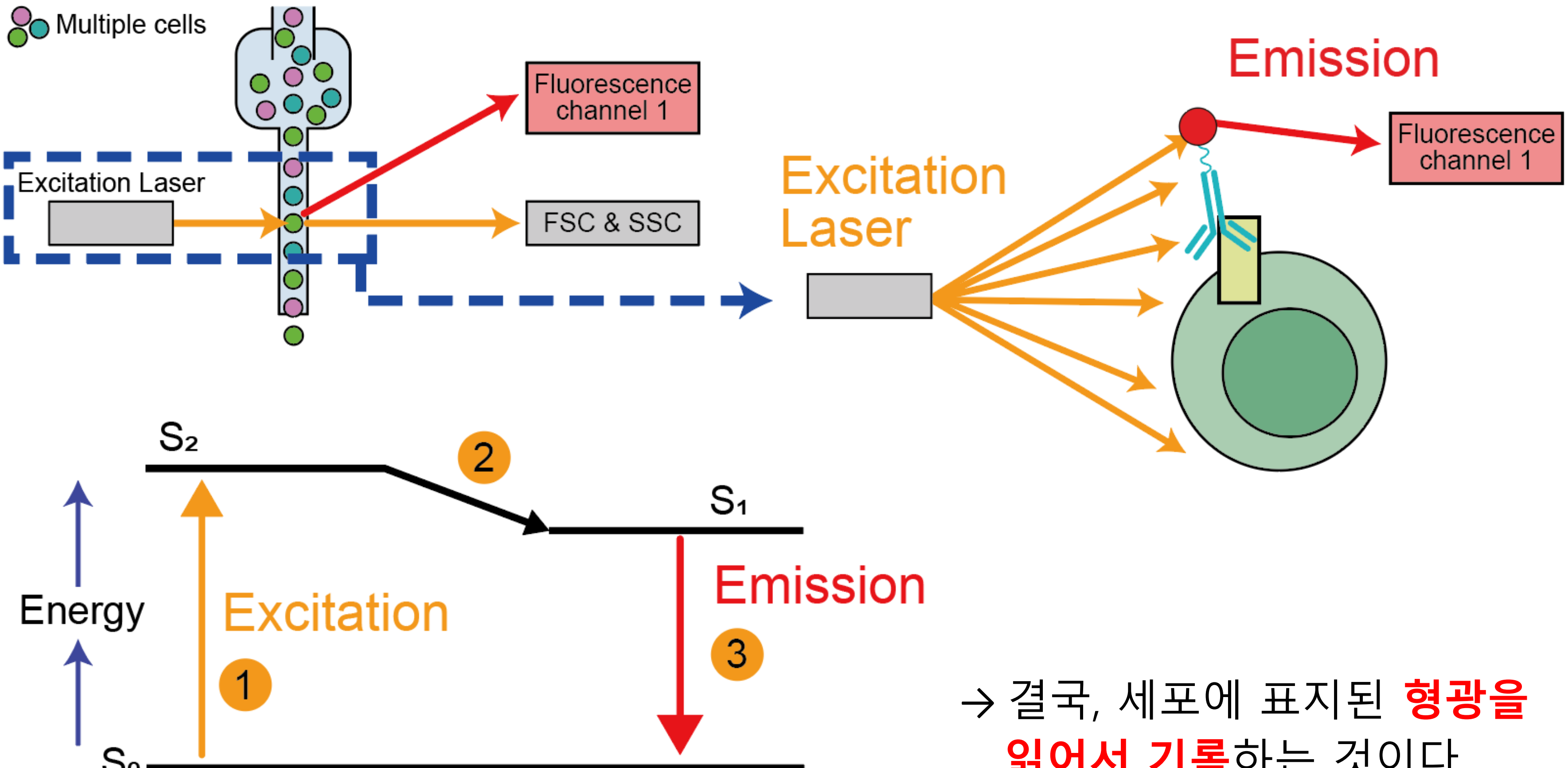
유세포 분석의 작동 원리 – Cell Labeling



Cell labeling for
Flow cytometry analysis

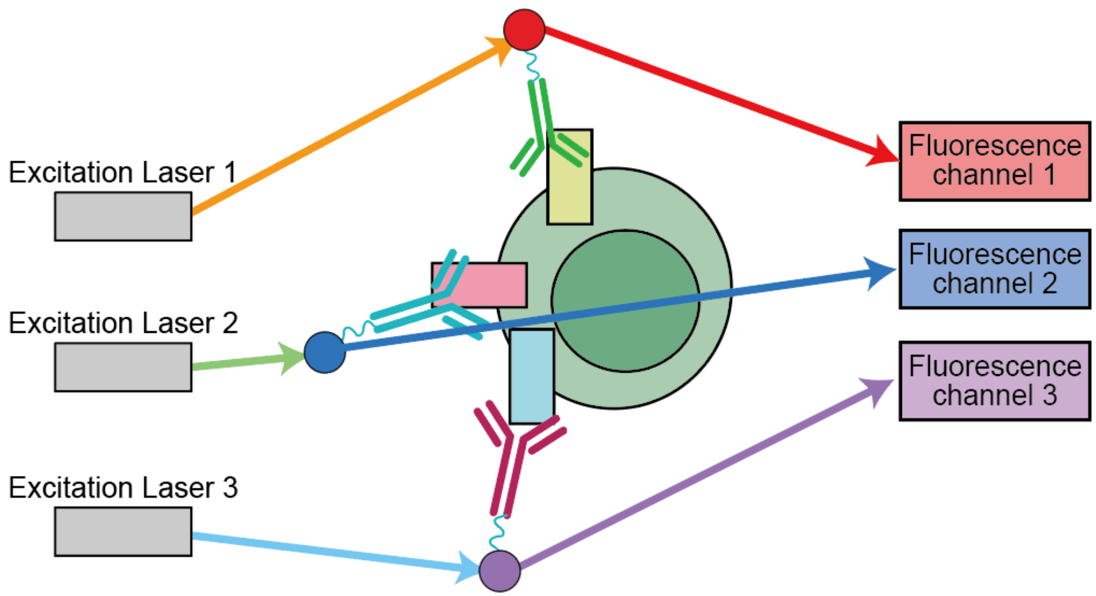
→ 세포들은 각 항원에 대해 (주로)
형광이 결합된 항체로 표지된다.

유세포 분석의 작동 원리 – Excitation & Emission

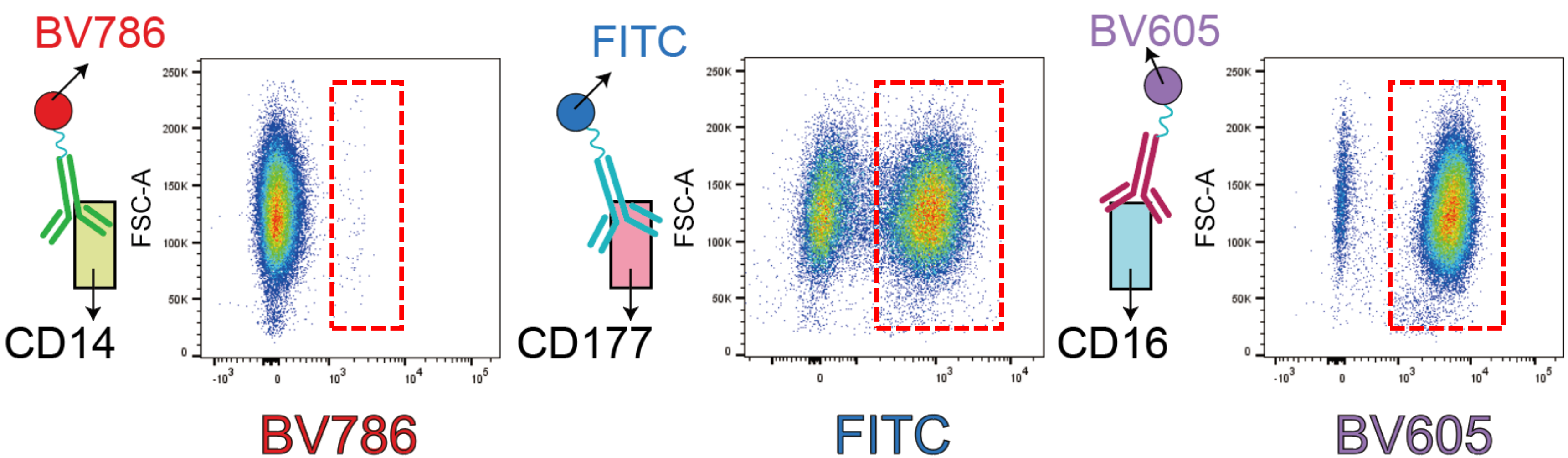


→ 결국, 세포에 표지된 **형광을 읽어서 기록**하는 것이다.

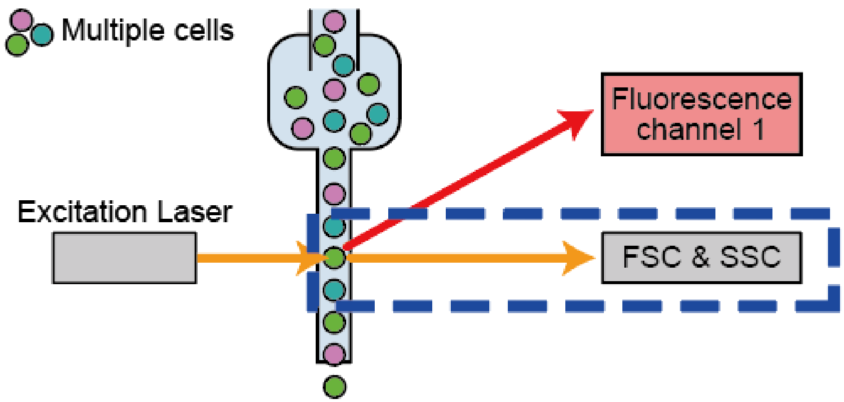
유세포 분석의 작동 원리 – Multiple Fluorochrome

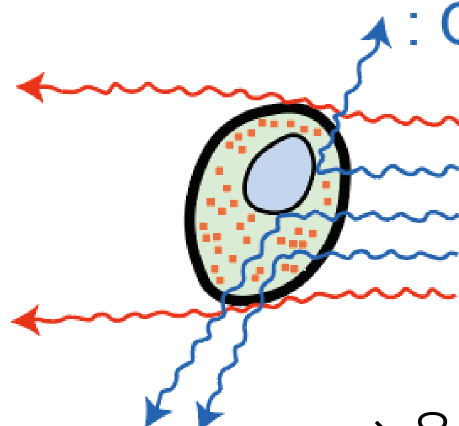


세포가 발현하는 여러가지 항원에 대해 **각기 다른 형광이 결합된 항체를 표지**하여, **각각의 형광을 서로 다른 채널**에서 값을 읽어내 나타낸다.



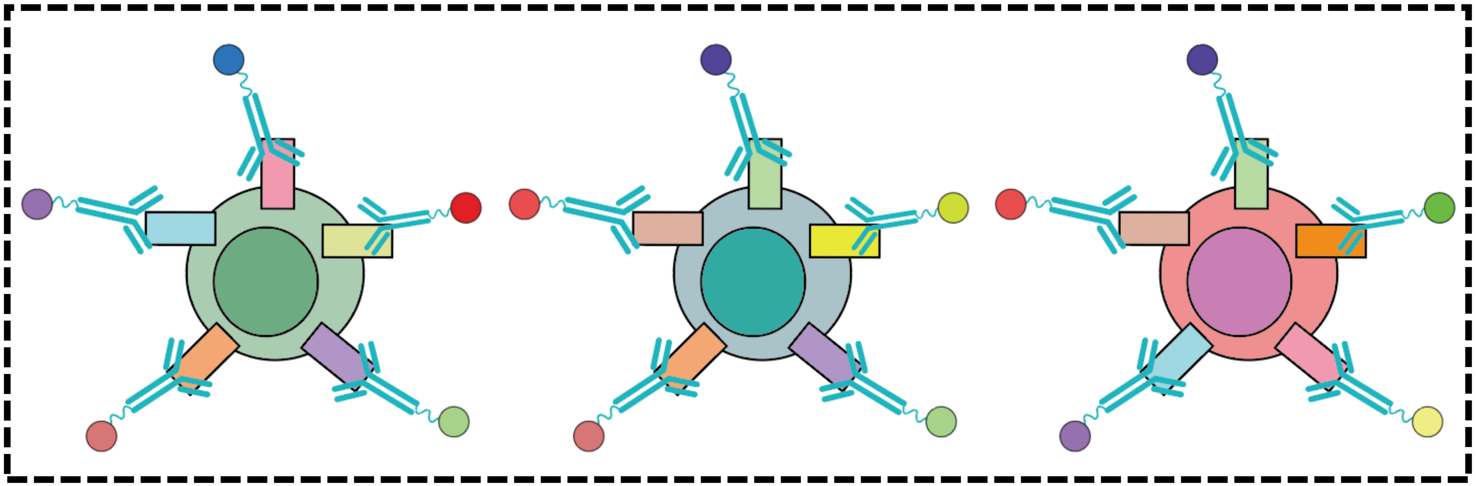
유세포 분석의 작동 원리 – Morphologic Gating



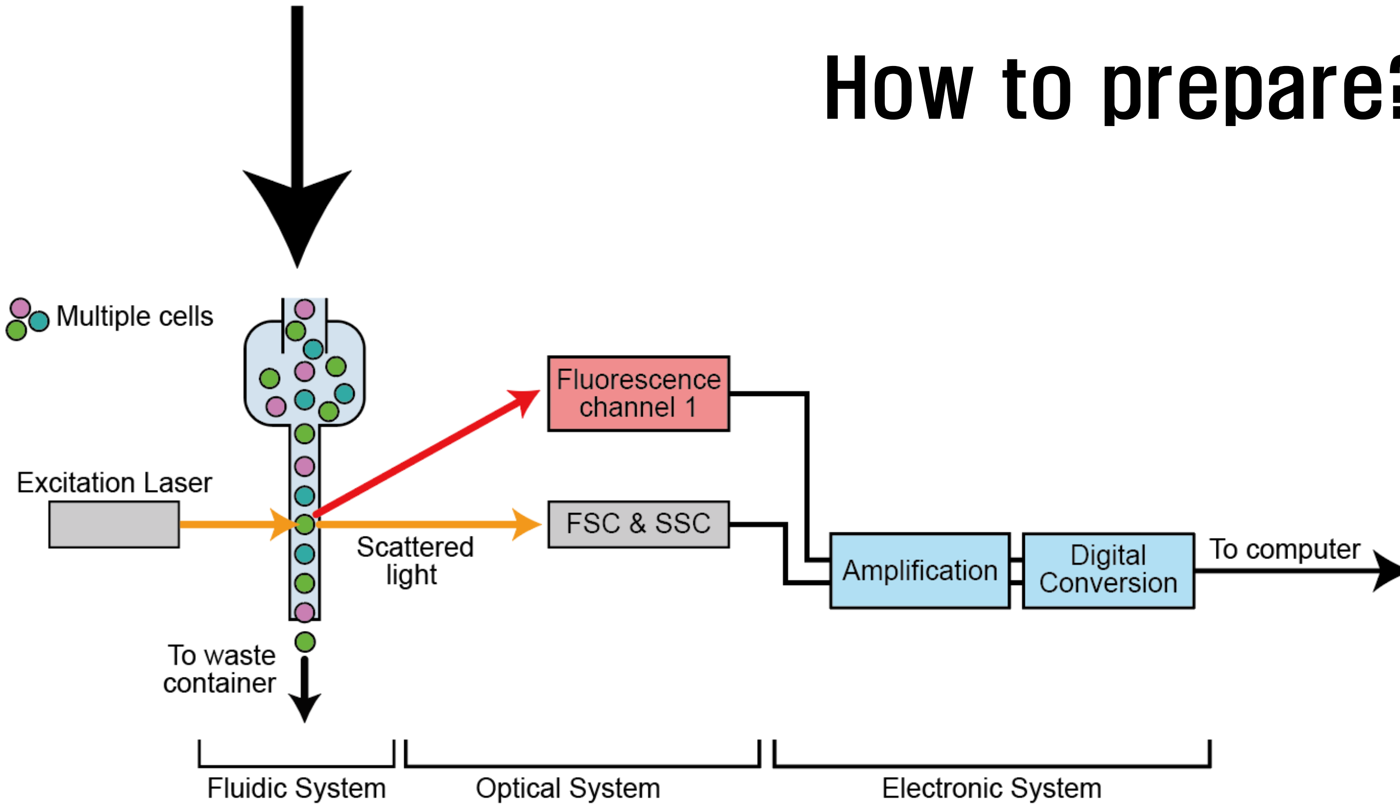


→ 유세포 분석은 **세포의 크기(FSC)와 복잡성(SSC)**을 활용하여 형태학적 구분이 가능하다.

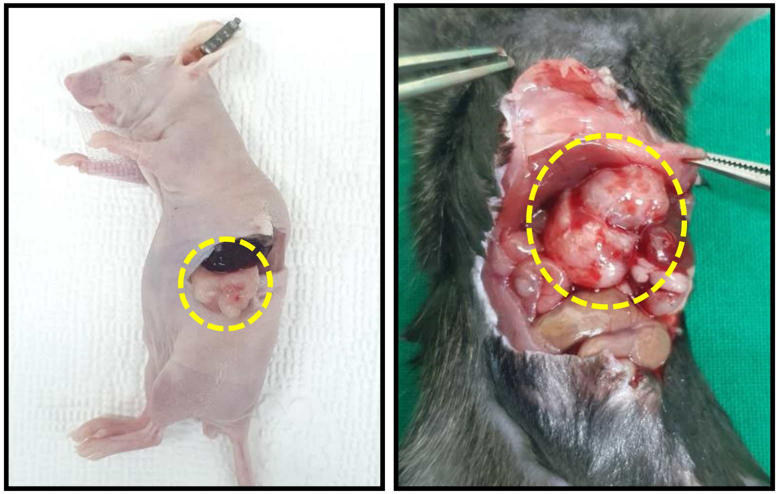
유세포 분석을 위한 세포 준비 과정

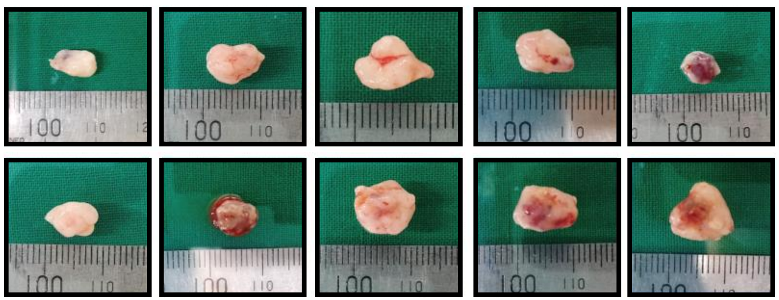


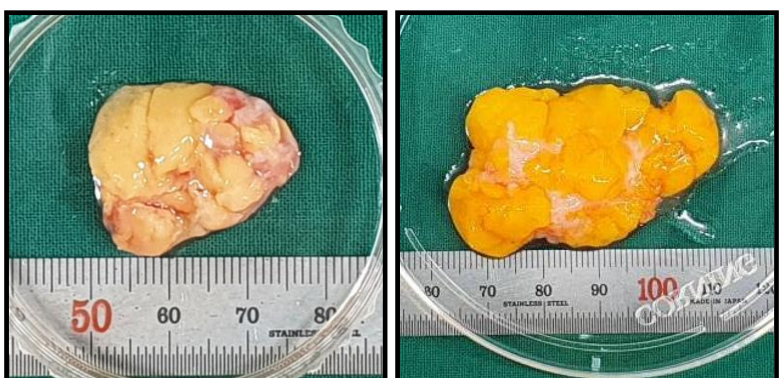
How to prepare?

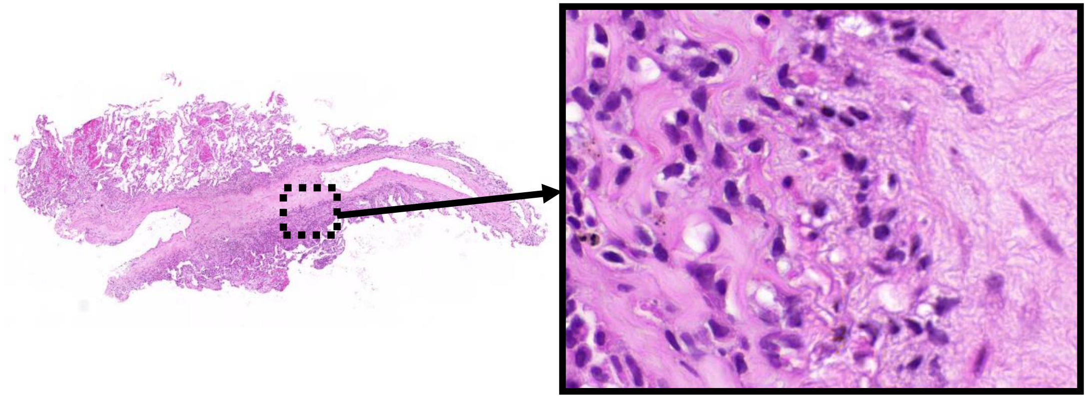


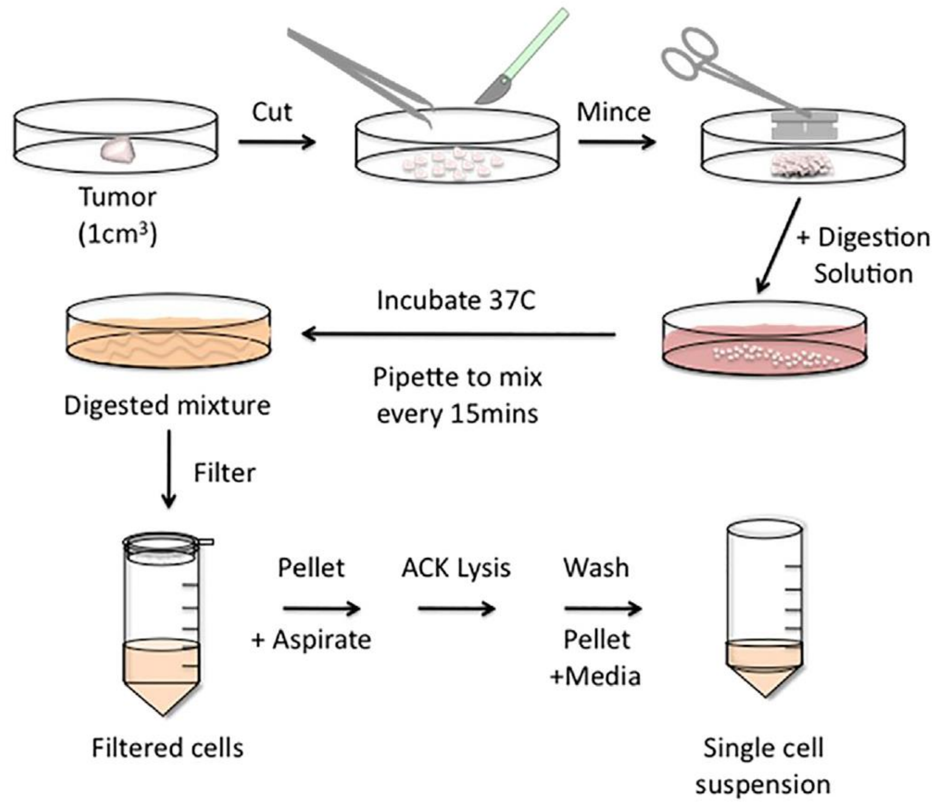
유세포 분석을 위한 세포 준비 – 조직(tissue)





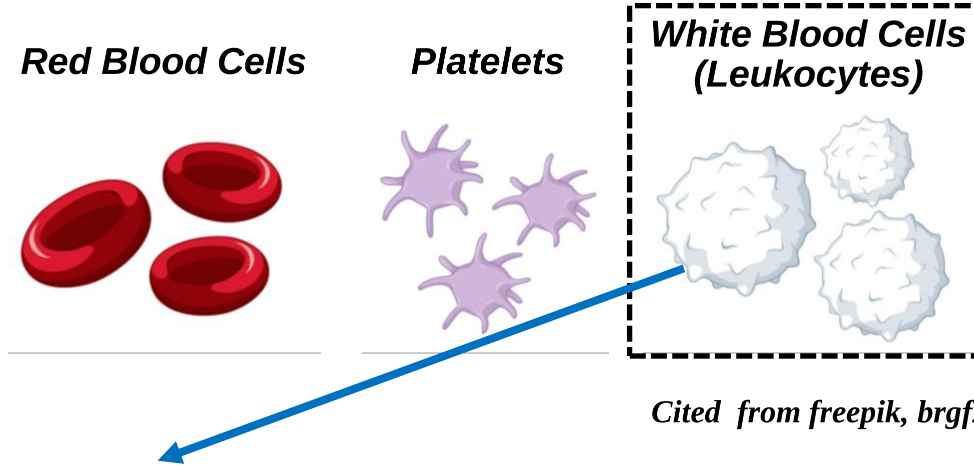






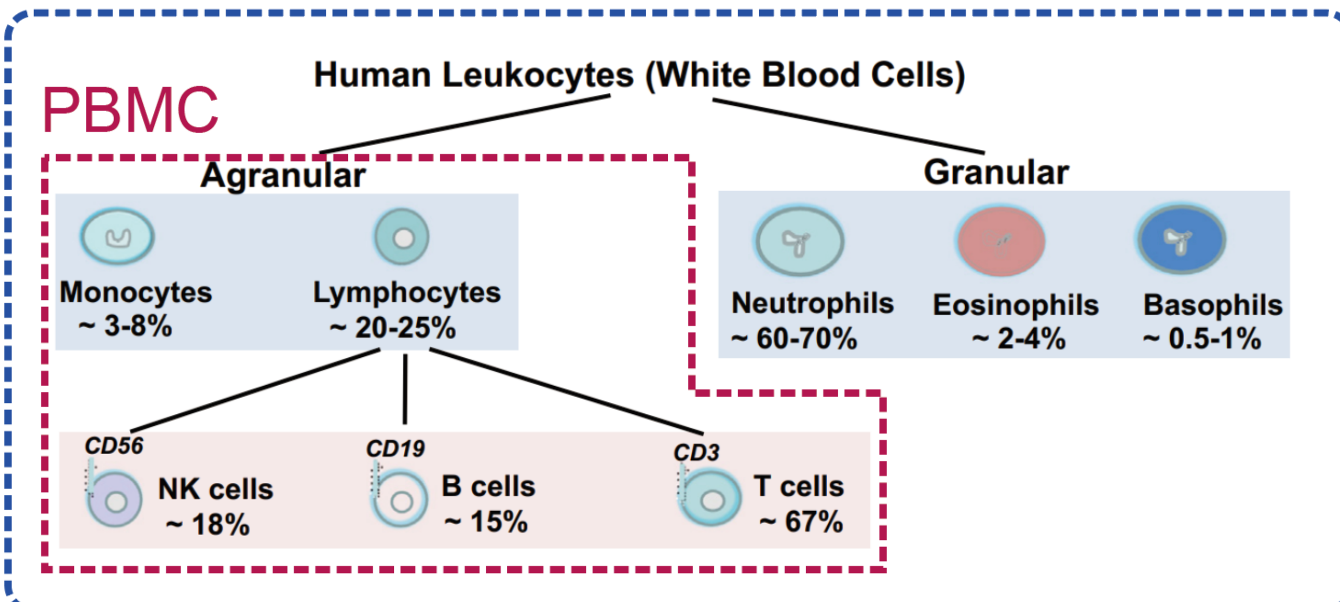
Drescher et al., 2021, biomedicines

유세포 분석을 위한 세포 준비 – 혈액(blood)



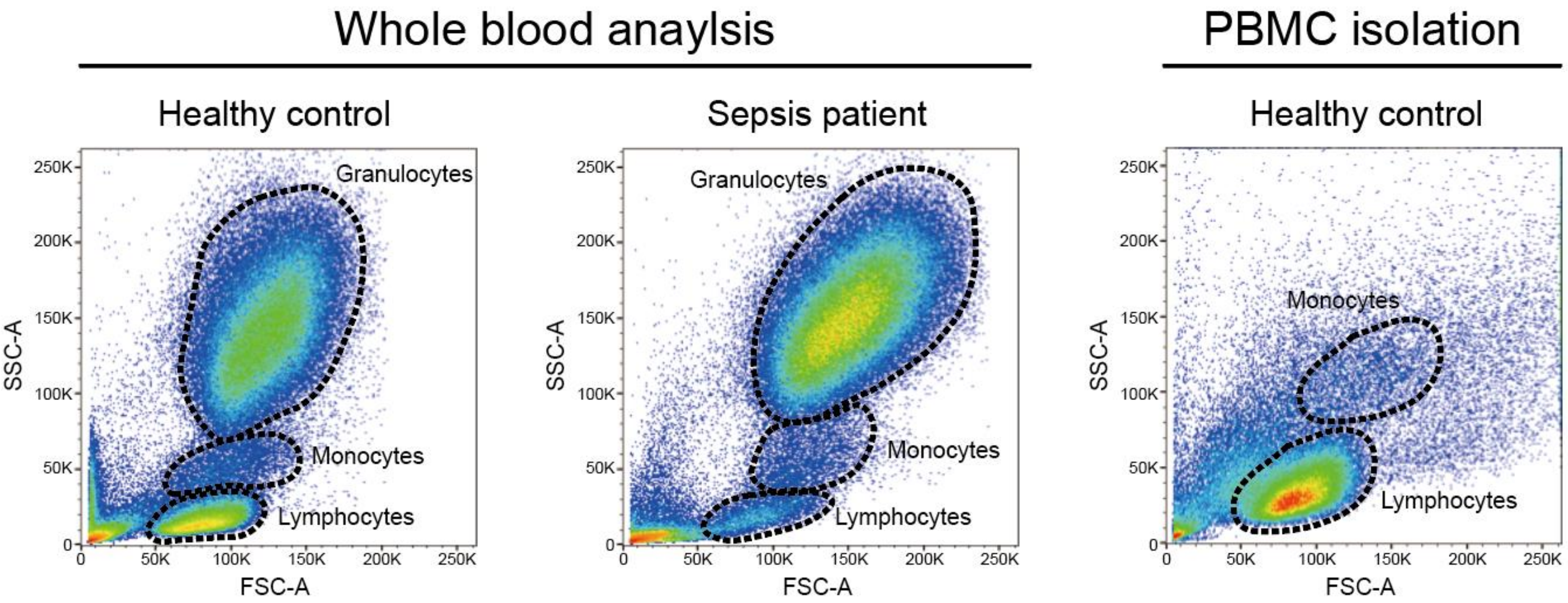
Cited from freepik, brgfx

Whole blood analysis

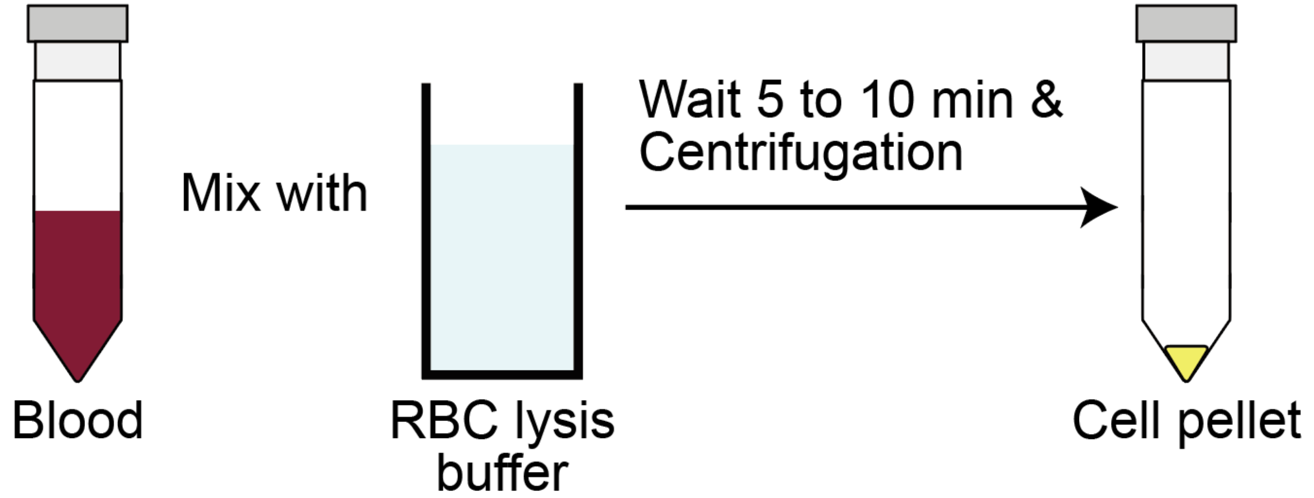


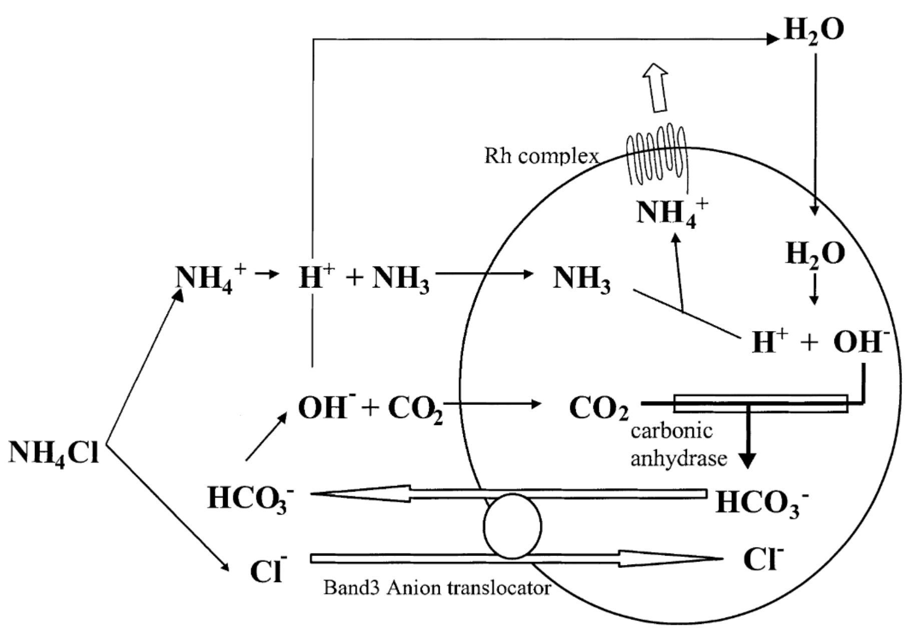
Cited from Personalized Epigenetics, 2015

Whole blood vs PBMC



Whole blood preperation





Drescher et al., 2021, biomedicines

PBMC preperation

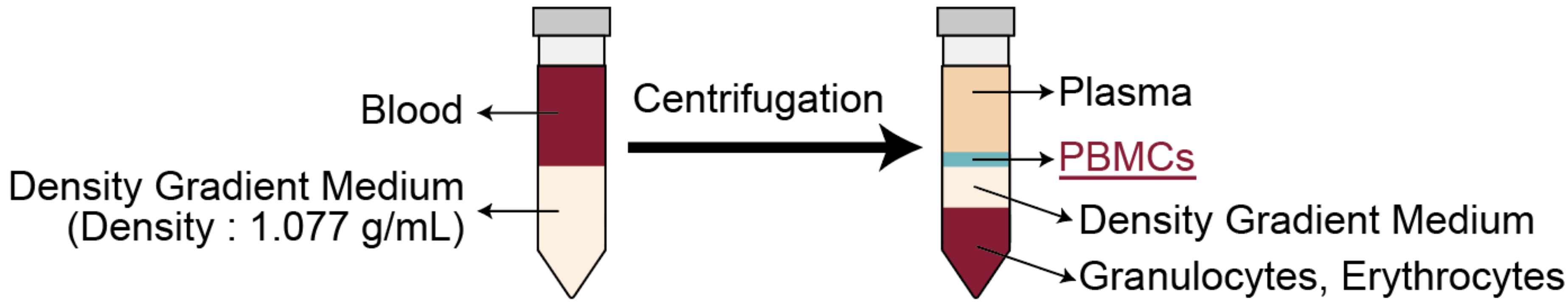


Table 1. The specific densities and effective radii of common blood components.

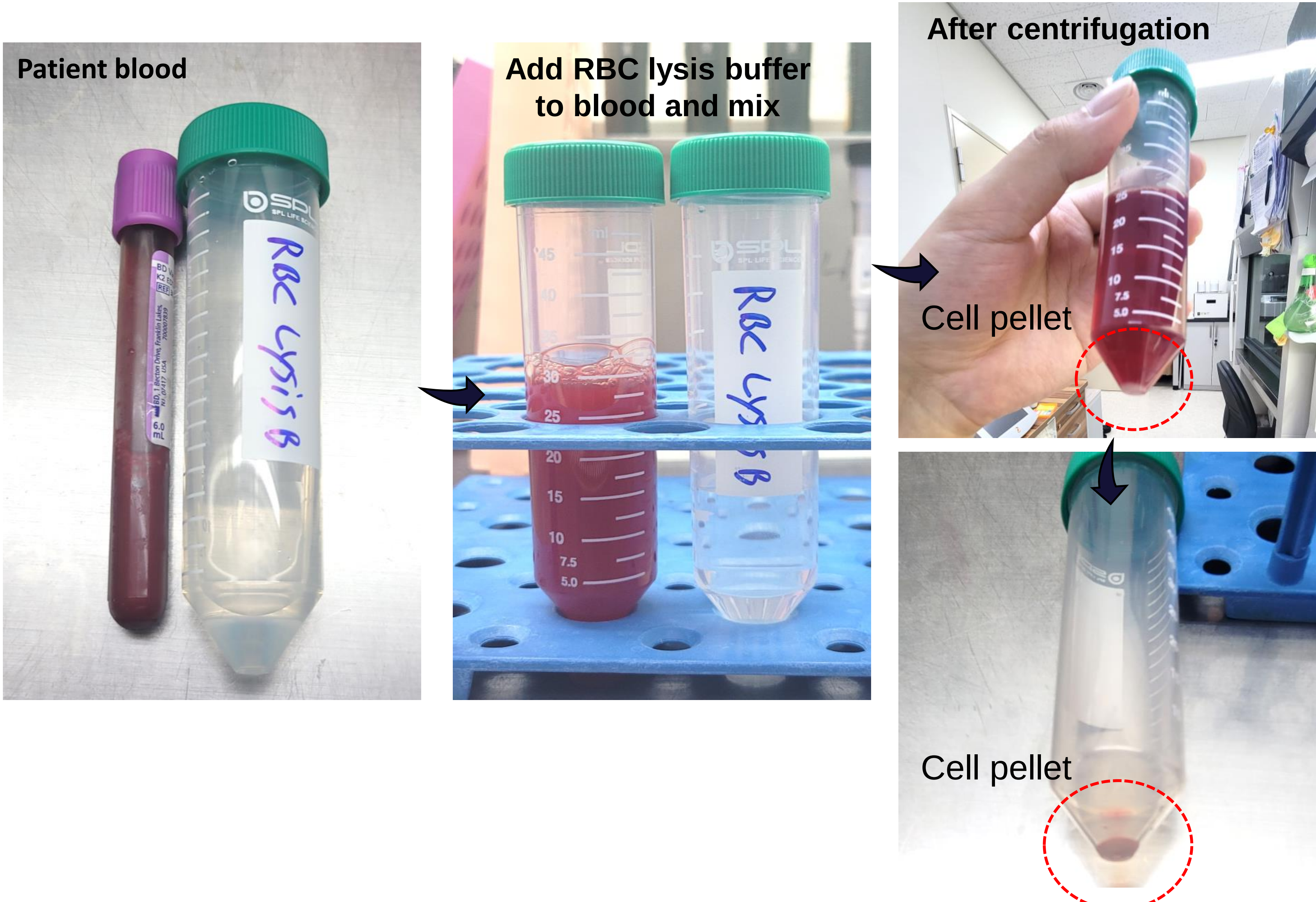
Blood cell type	*Specific density(g/mL)*	*Effective radius (μm)*
Monocyte	1.067–1.077[22]	6–7.5[23]
Lymphocyte	1.073–1.077[22]	3–6[23]
Basophil	1.072–1.078[24]	4.5–5[23]
Neutrophil	1.08–1.09[25]	6–7.5[23]
Eosinophil	1.09–1.1[25]	6–7.5[23]
RBC	1.098[26]	2.63 (oblate spheroid)

Moen et al., 2016, PLOS ONE

PBMC preparation – Real practice

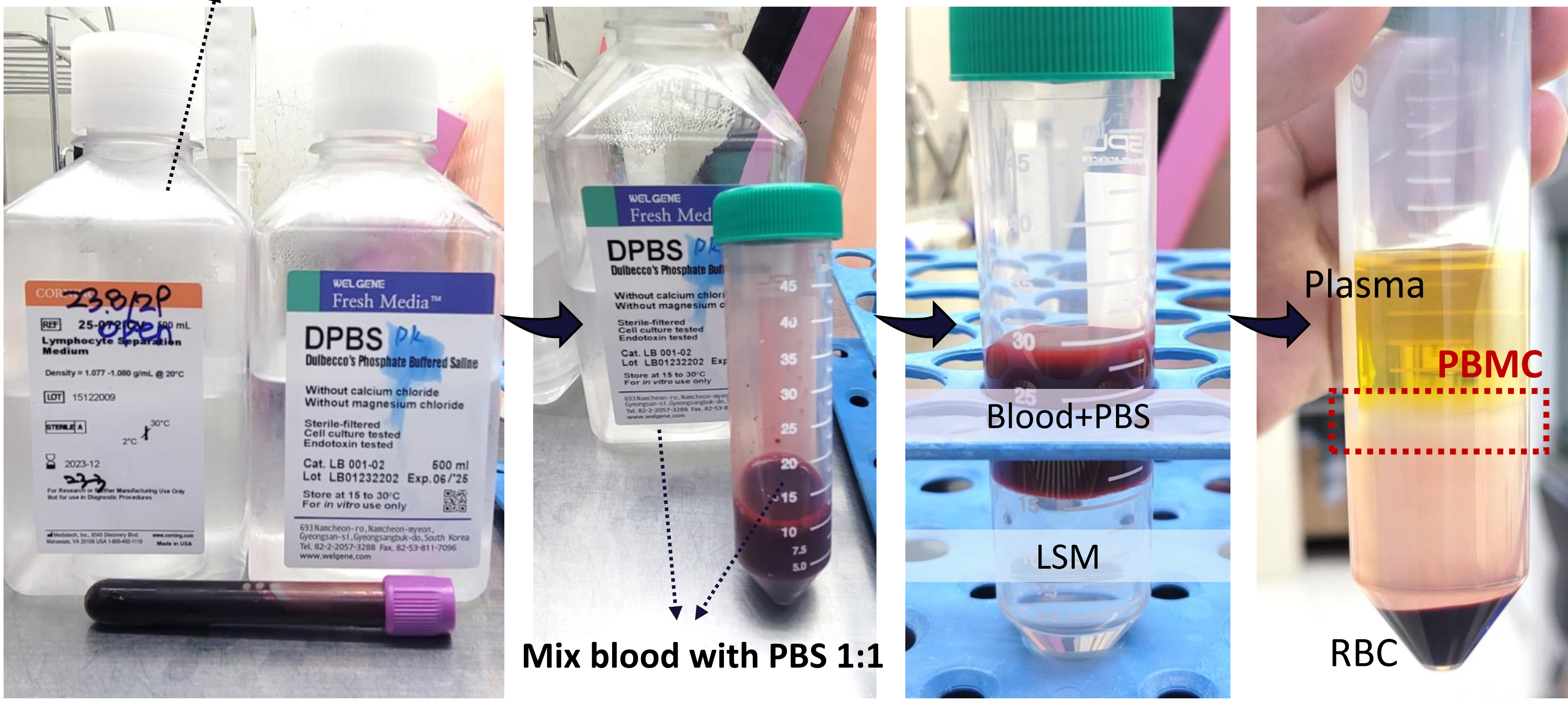
Cell culture hood

Real practice – Whole Blood Lysis

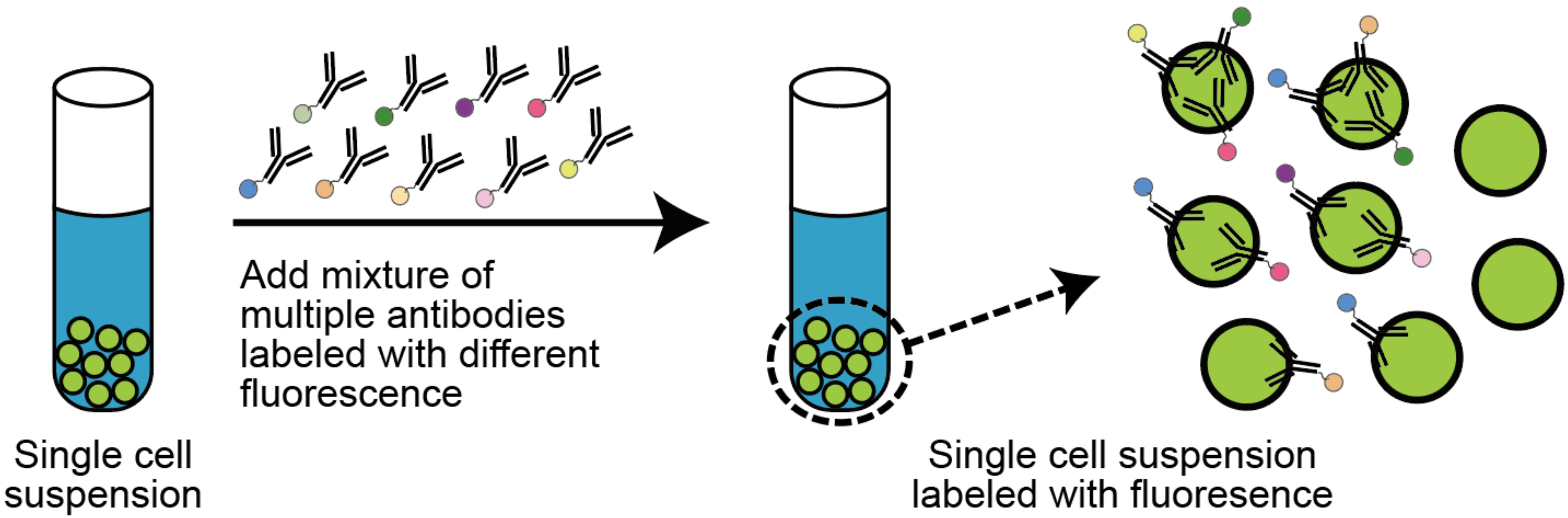


Real practice – PBMC isolation

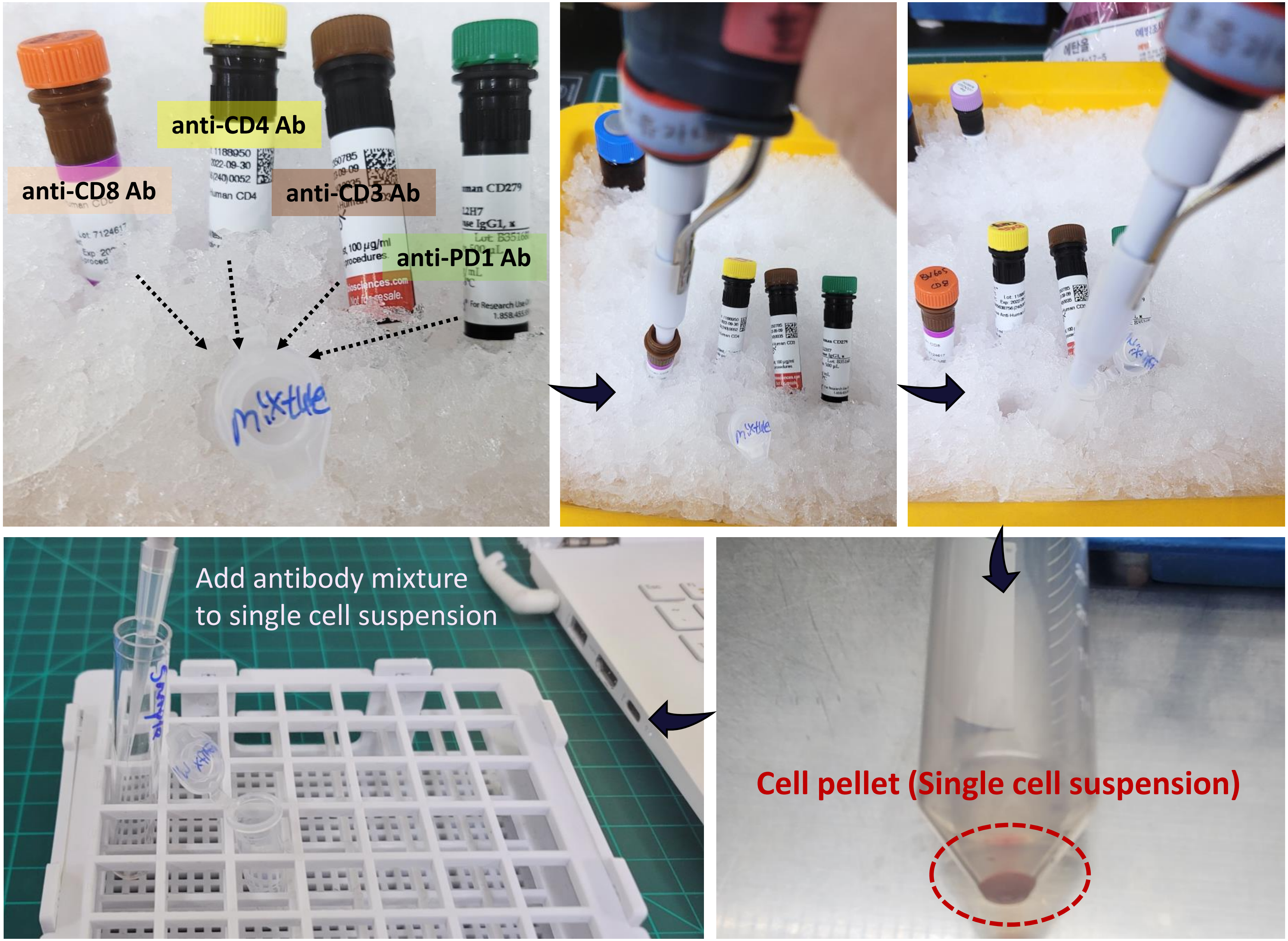
Density gradient medium
(Lymphocyte separation medium, **LSM**)



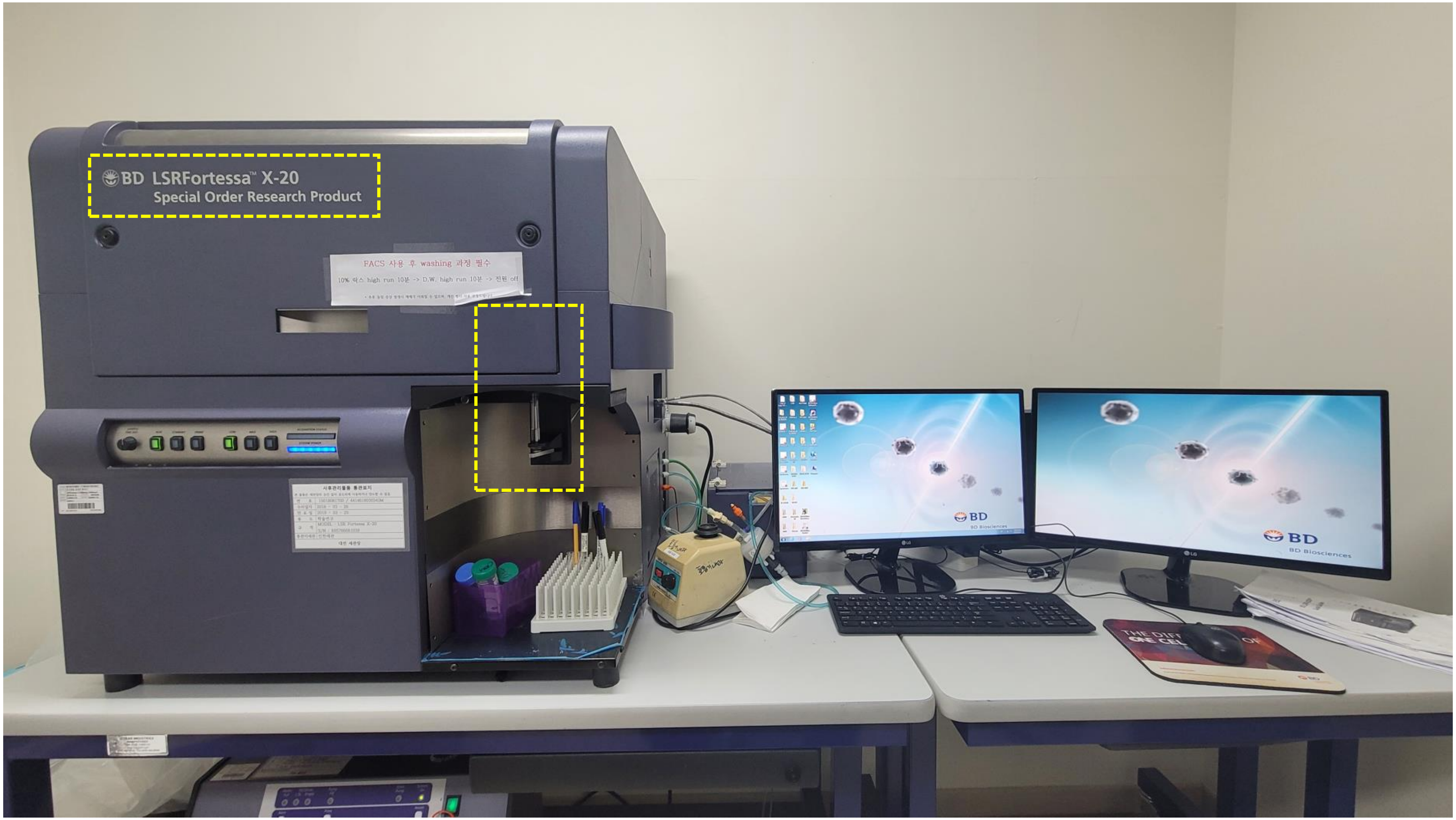
FACS staining



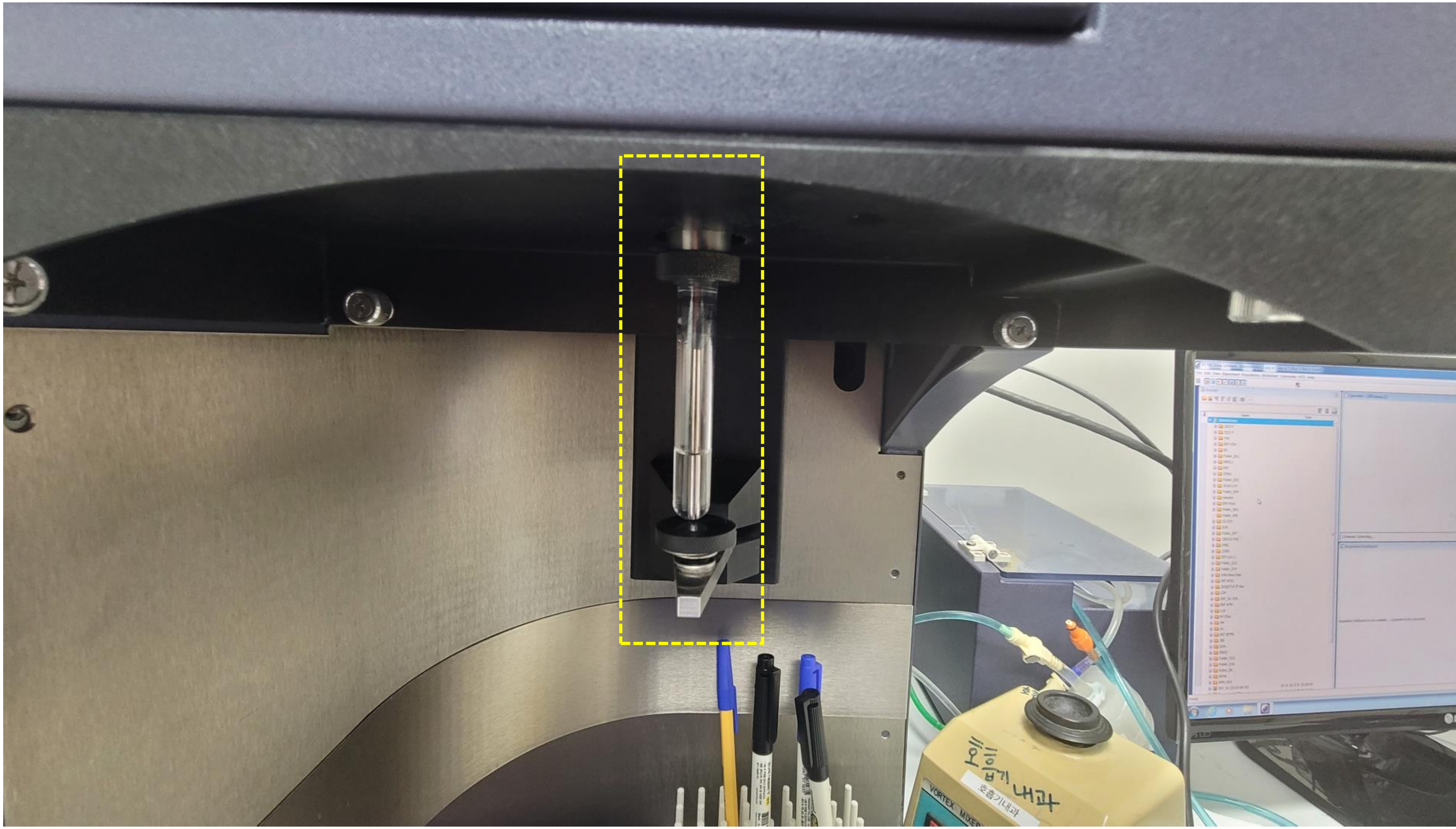
FACS staining



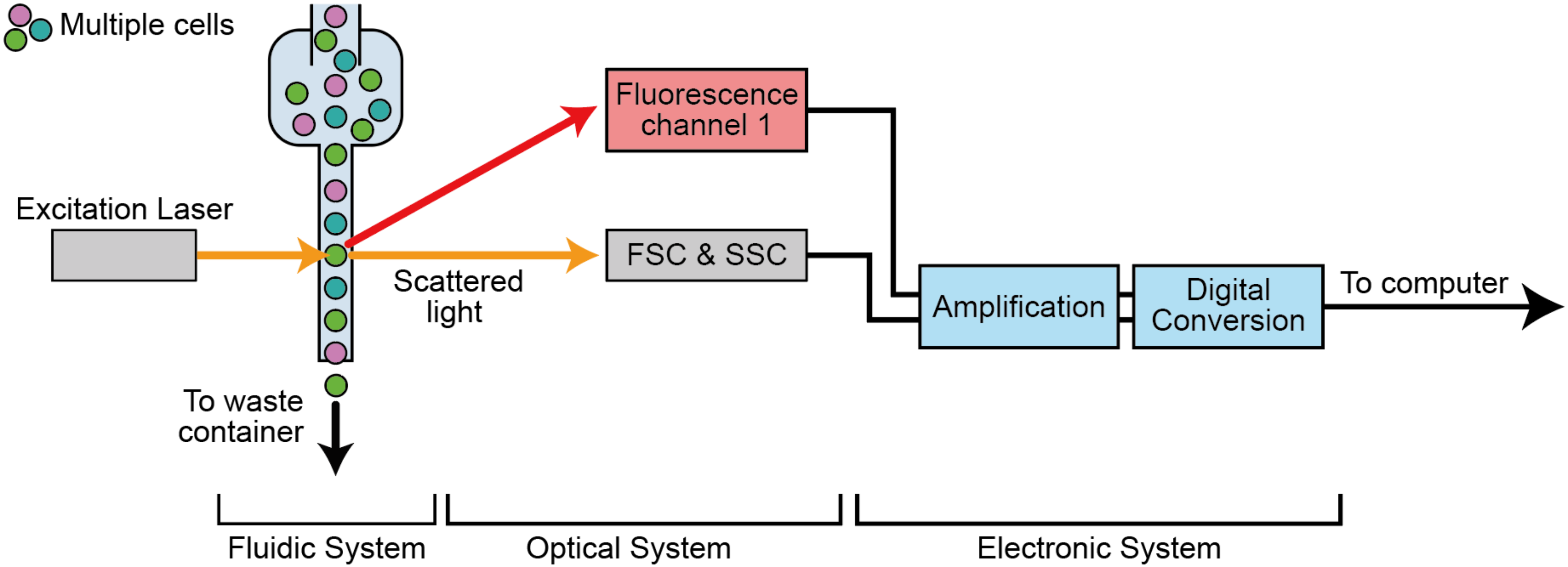
유세포 분석 기계


BD LSRFortessa™ X-20
Special Order Research Product
FACS 사용 후 washing 과정 필수
BD
BD Biosciences

유세포 분석 기계



유세포 분석 기계



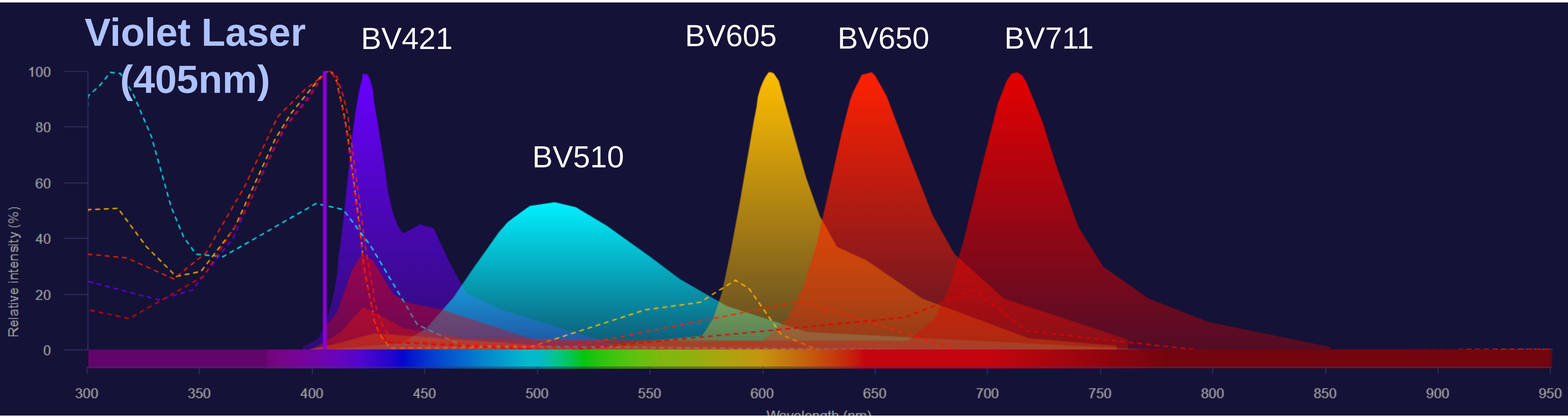
	Violet Laser (405nm)				
	BV421	BV510	BV605	BV650	BV711
Molecule			CD8	CD4	

	Blue laser (488nm)				
	FITC	PE	PE-TR	PerCP-Cy5.5	PE-Cy7
Molecule	PD-1				

	Red laser (640nm)		
	APC	A700	APC-Cy7
Molecule			CD3

유세포 분석 기계

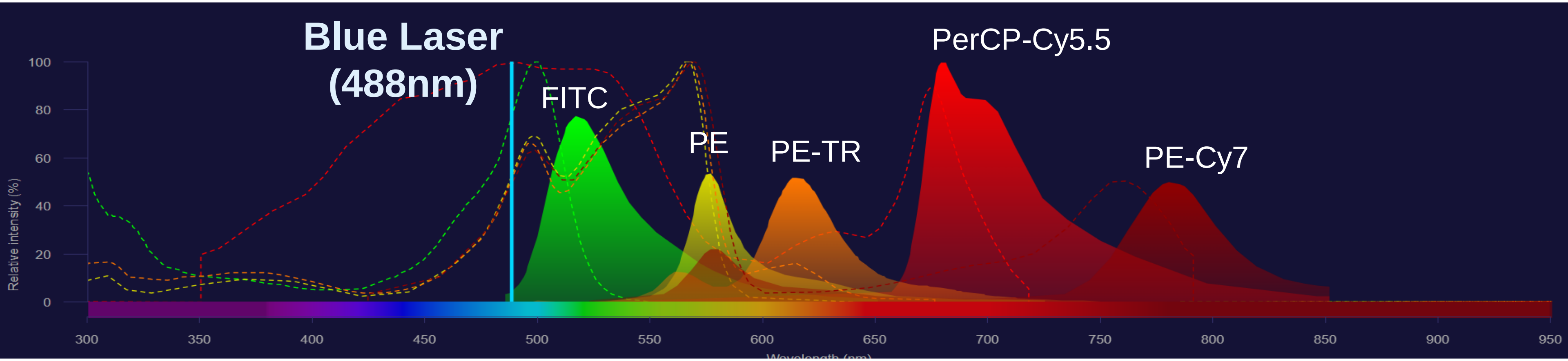
	Violet Laser (405nm)				
	BV421 (421nm)	BV510 (512nm)	BV605 (602nm)	BV650 (647nm)	BV711 (711nm)
Molecule			CD8	CD4	



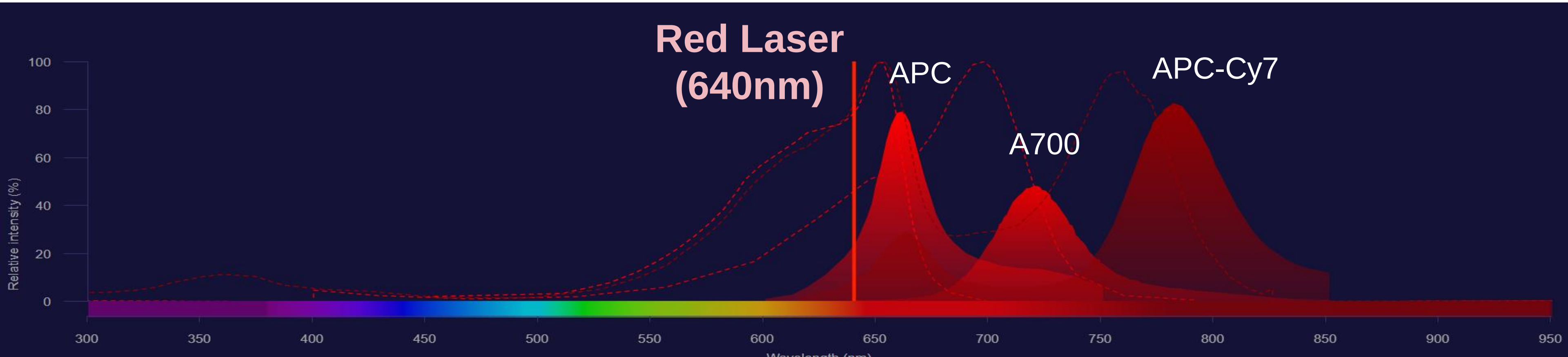
→ Violet Laser에 의해 excitation 되어 602nm에서 maximum emission을 보이는 형광이 결합된 **CD8**에 결합하는 항체와,
Violet Laser에 의해 excitation 되어 647nm에서 maximum emission을 보이는 형광이 결합된 **CD4**에 결합하는 항체를 사용해 유세포 분석을 하겠다.

유세포 분석 기계

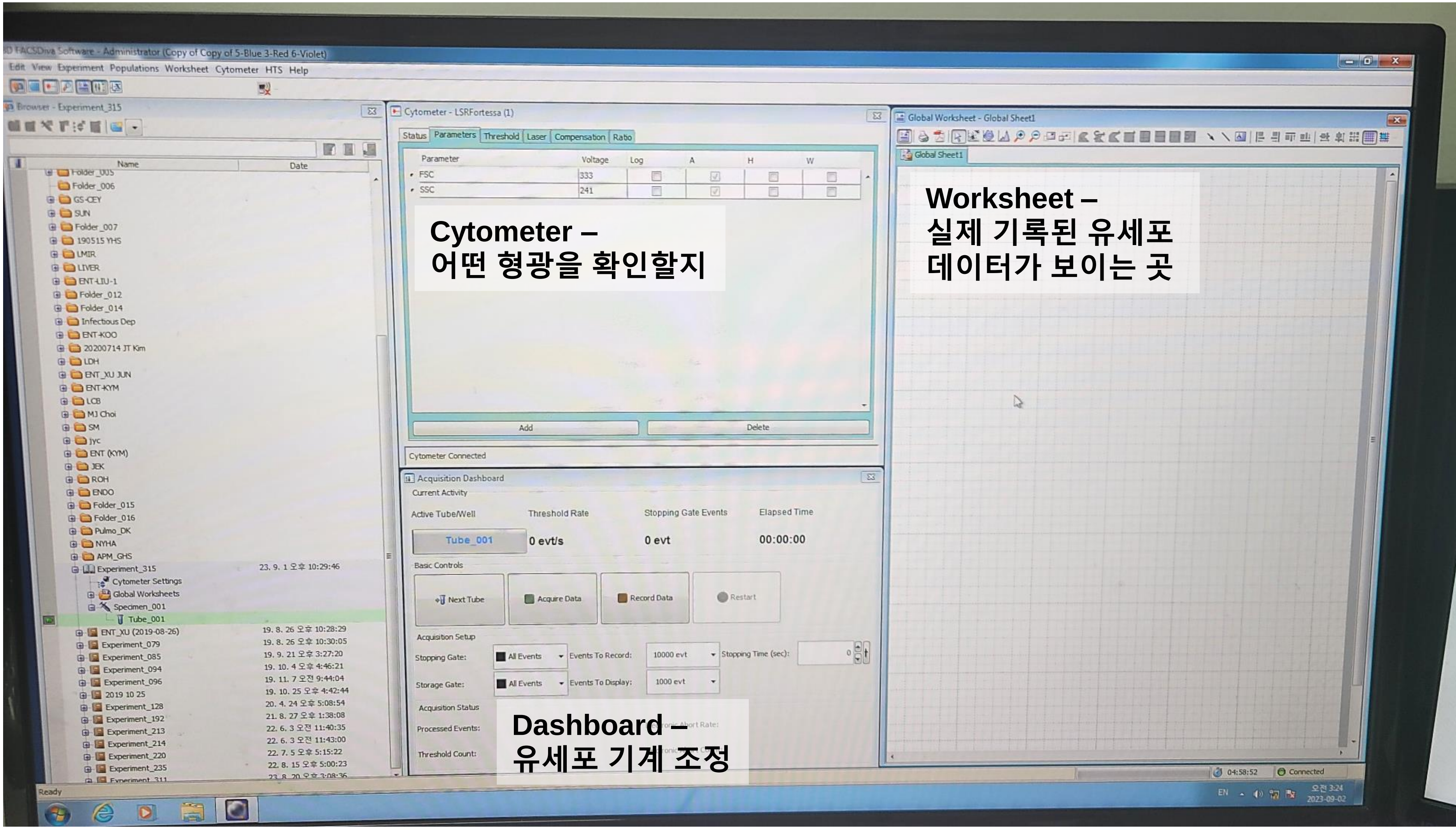
	Blue laser (488nm)				
	FITC (516nm)	PE	PE-TR	PerCP-Cy5.5	PE-Cy7
Molecule	PD-1				



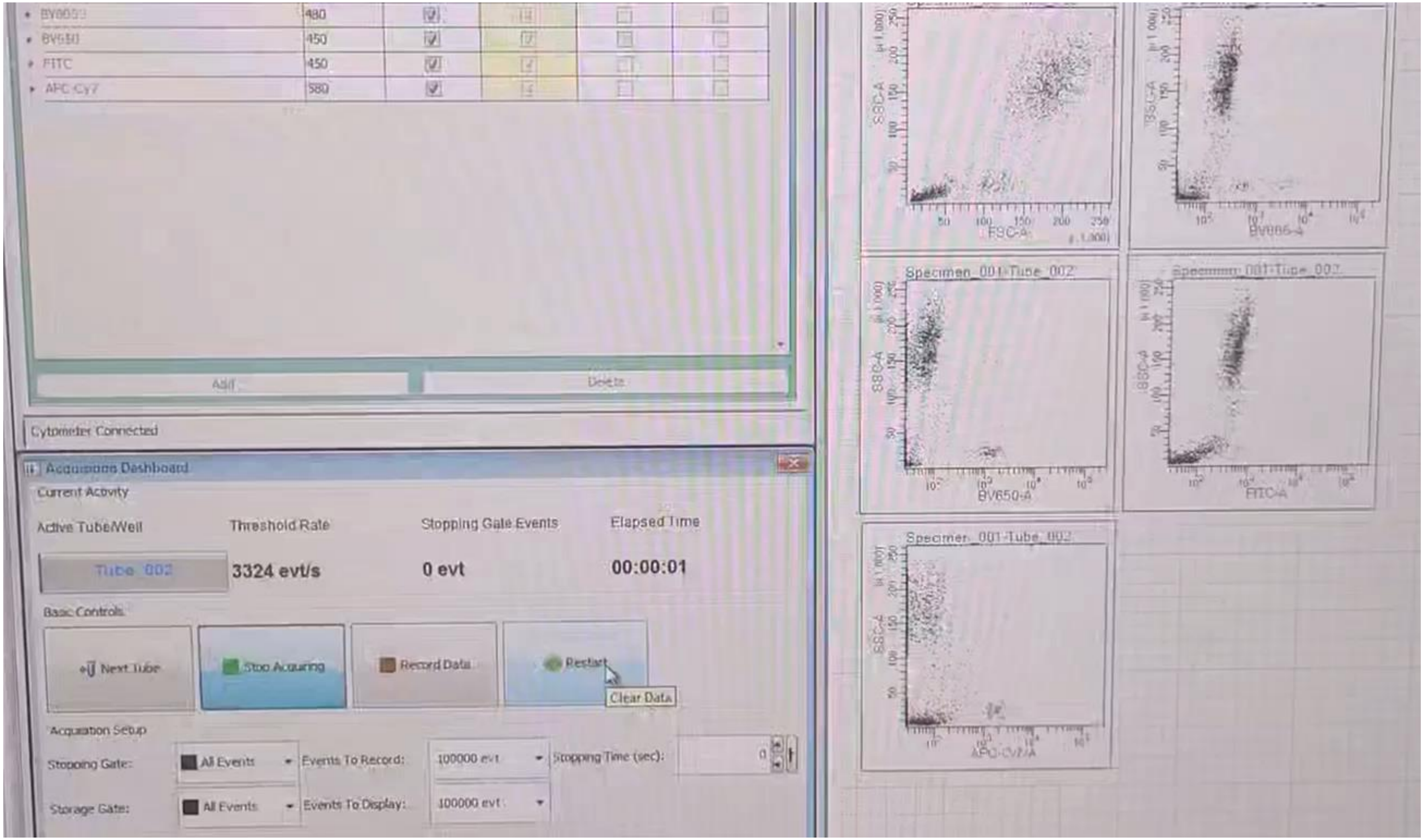
	Red laser (640nm)		
	APC	A700	APC-Cy7 (779nm)
Molecule			CD3



유세포 분석 기계

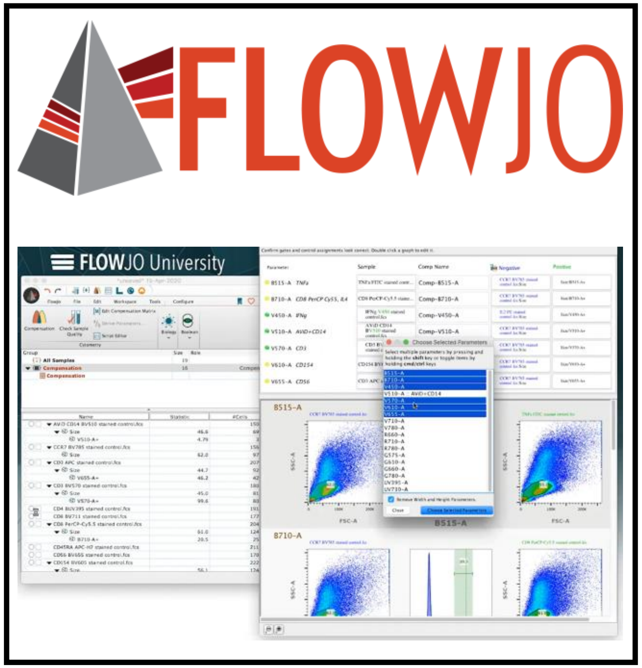

Cytometer –
어떤 형광을 확인할지
Worksheet –
실제 기록된 유세포
데이터가 보이는 곳
Dashboard –
유세포 기계 조정

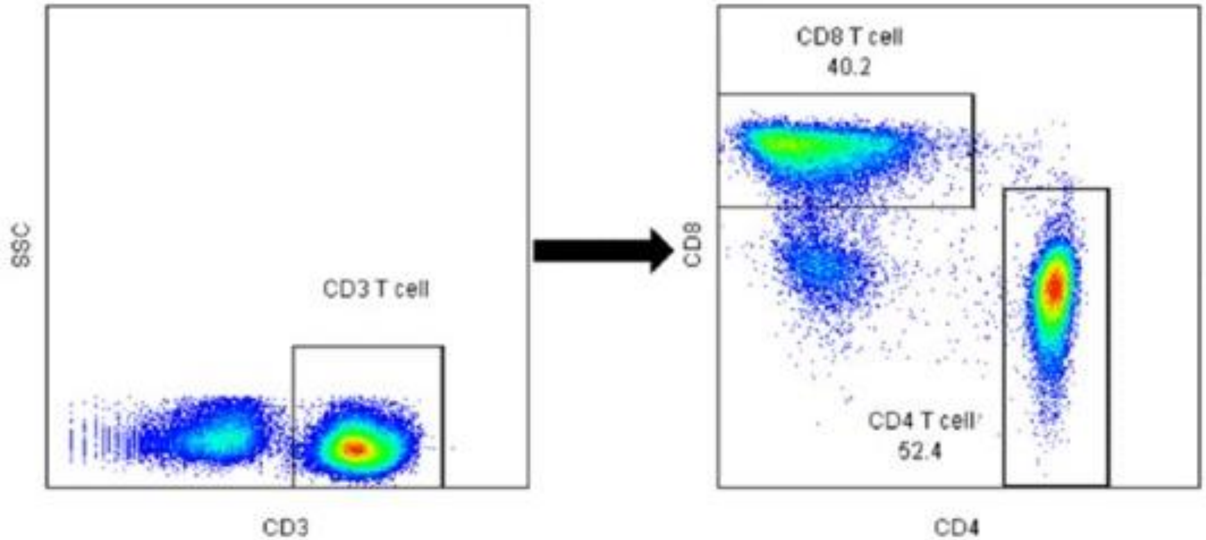
유세포 분석 기계



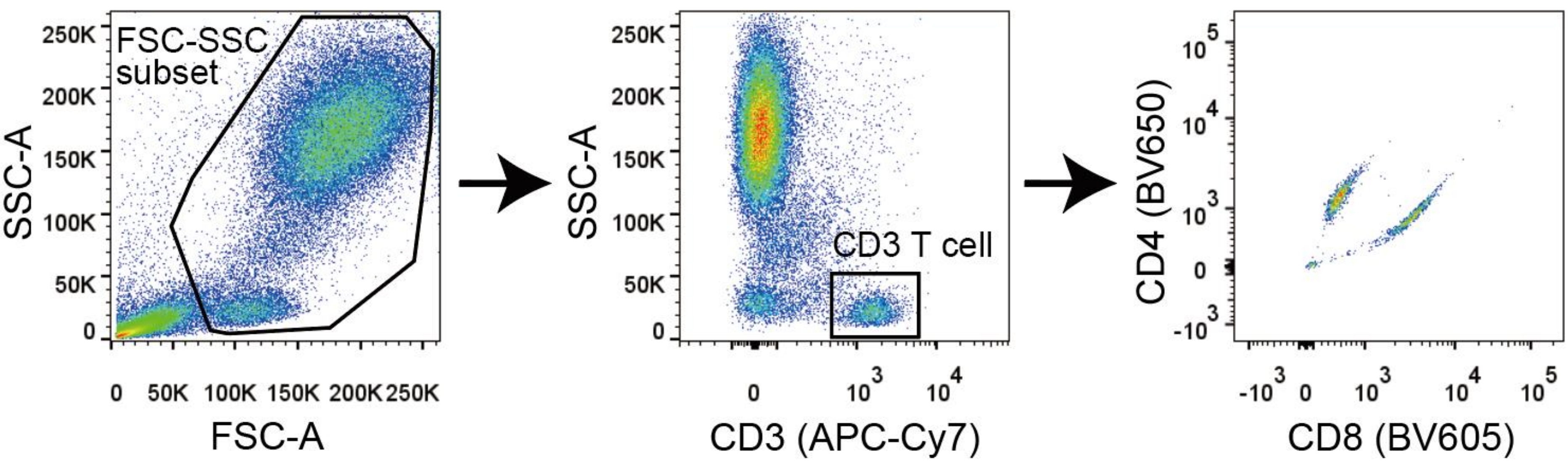
FITC
APC-Cy7
Add
Delete
Cytometer Connected
Acquisition Dashboard
Current Activity
Active Tube/Well
Threshold Rate
Stopping Gate Events
Elapsed Time
3324 evt/s
0 evt
00:00:01
Basic Controls
Next Tube
Stop Acquiring
Record Data
Restart
Clear Data
Acquisition Setup
Stopping Gate:
All Events
Events To Record:
100000 evt
Stopping Time (sec):
Storage Gate:
All Events
Events To Display:
100000 evt
Specimen_001-Tube_002
SSC-A
FSC-A
BV650-A
FITC-A
APC-Cy7-A

유세포 분석 기계



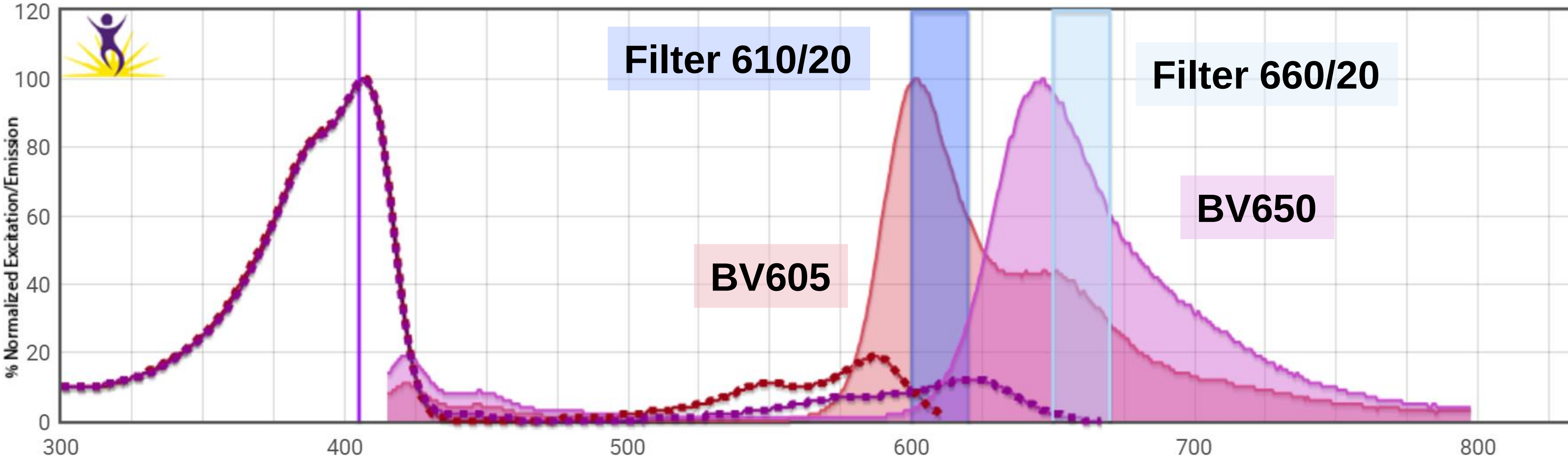


Xiaoyan et al., 2019, BMC Nephrology



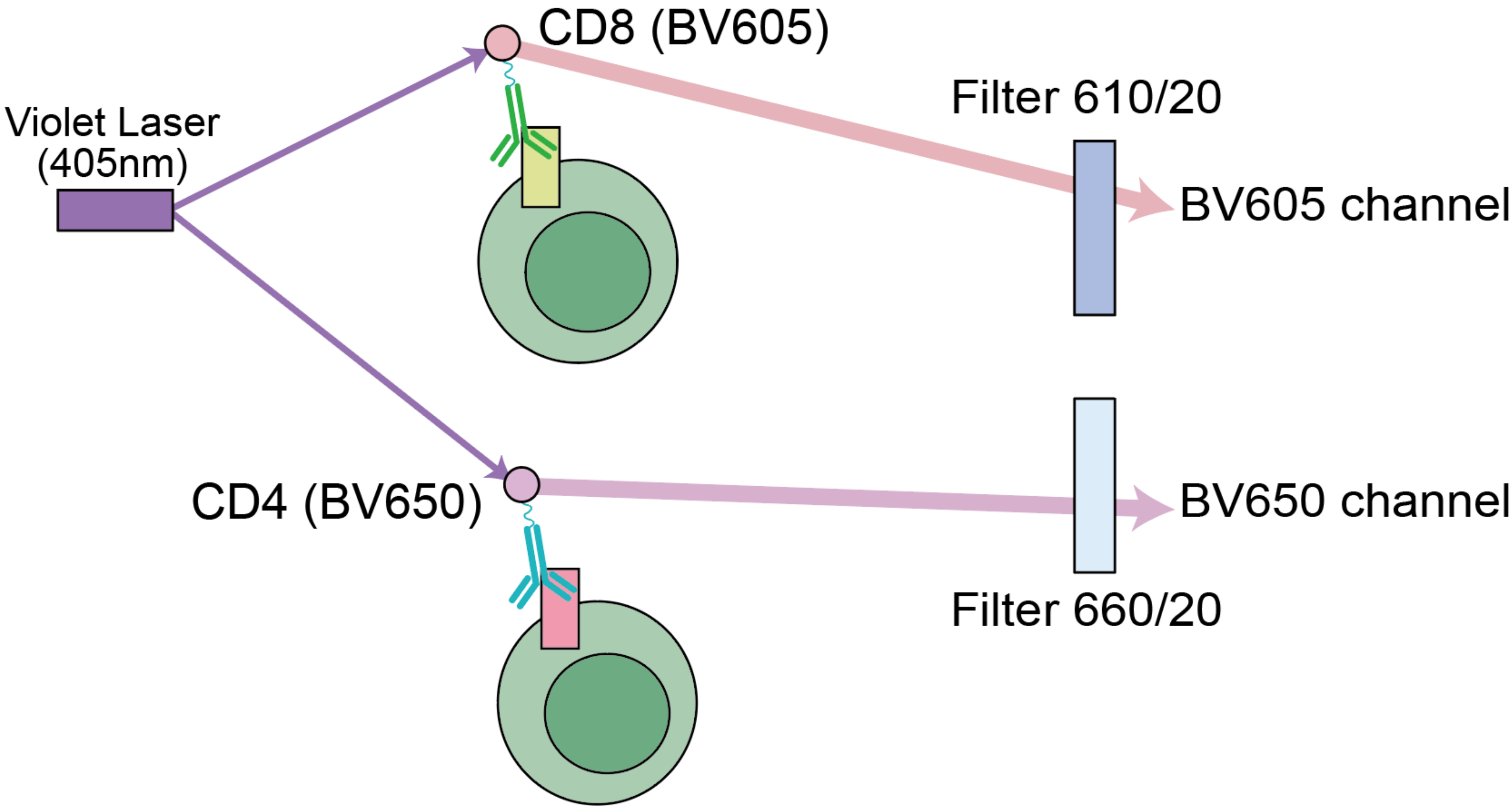
Compensation

	Violet Laser (405nm)				
	BV421	BV510	BV605	BV650	BV711
Molecule			CD8	CD4	

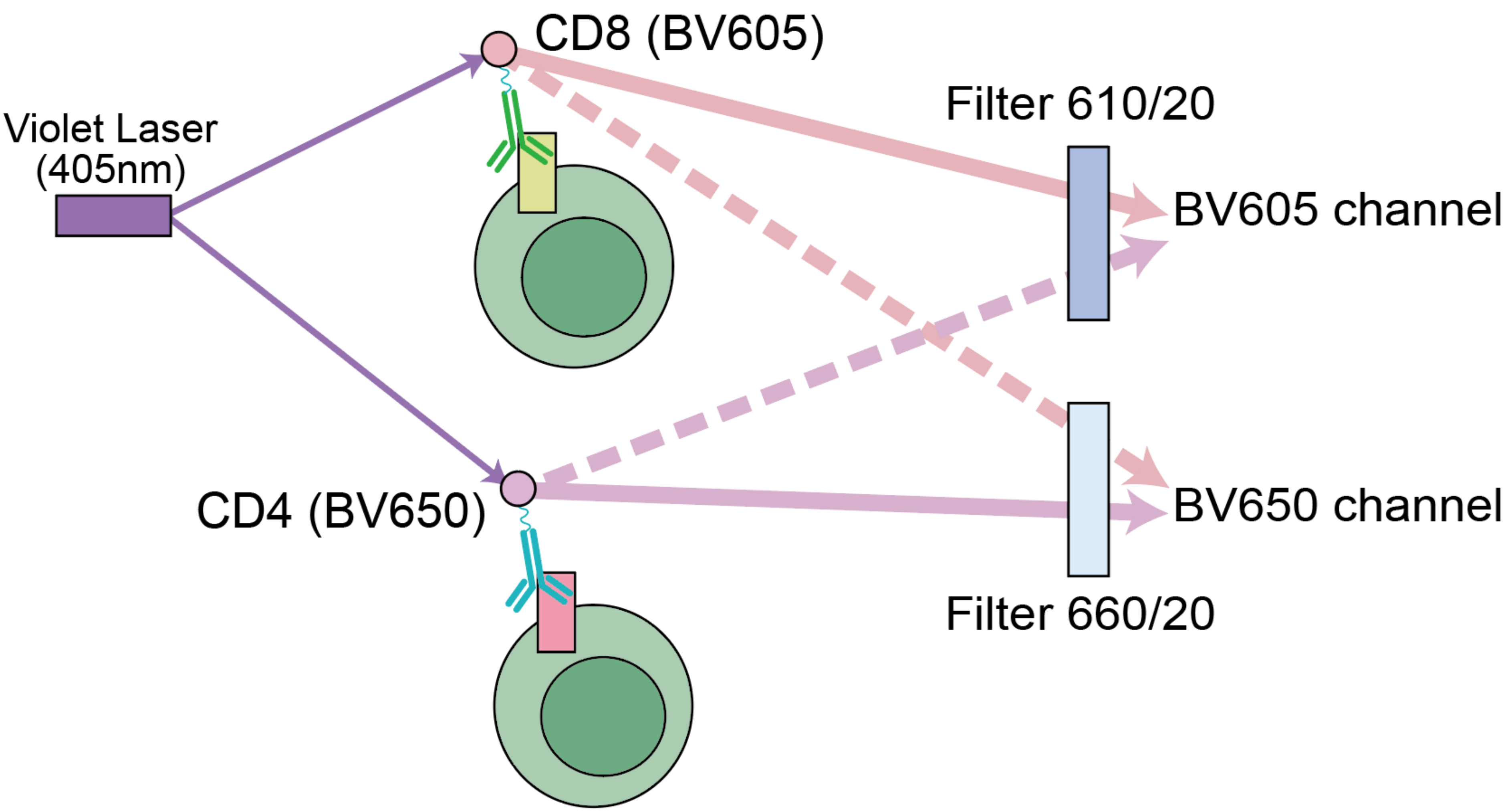


각 파장이 서로 침범하여 겹치는 현상을 **'Spill over'**라고 한다.
Spill over를 보정해주는 과정을 **Compensation** 이라 한다.

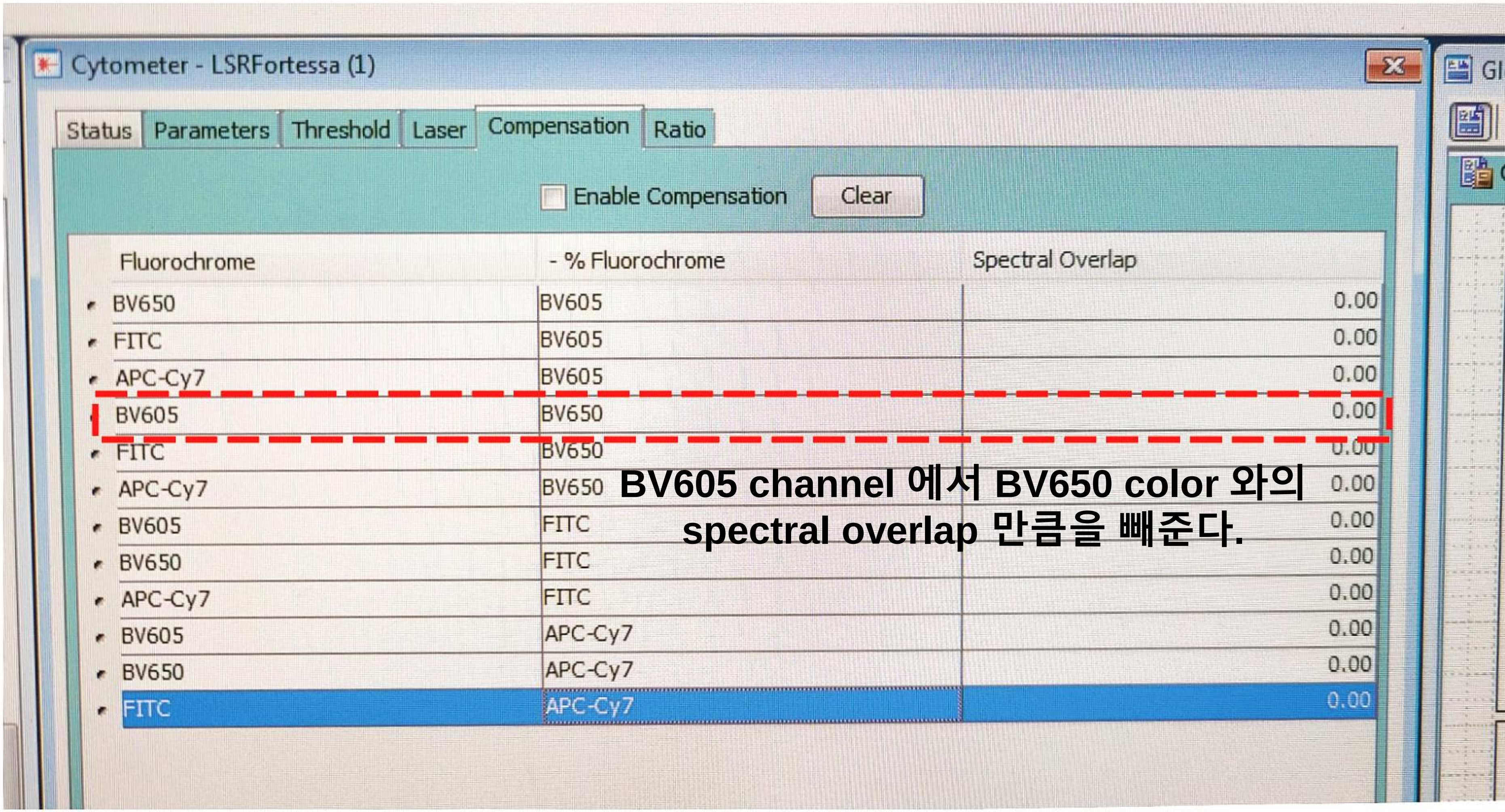
Compensation



Compensation

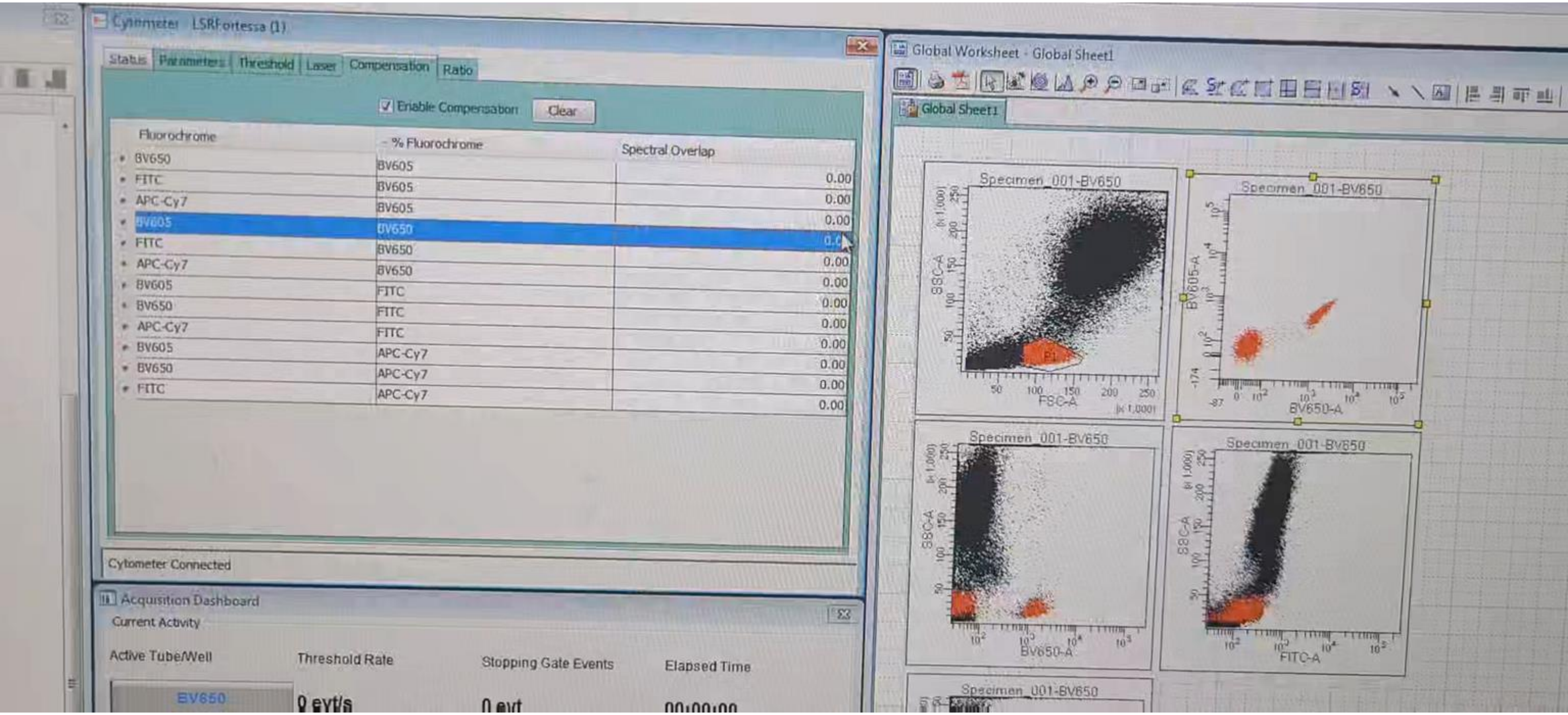


Compensation

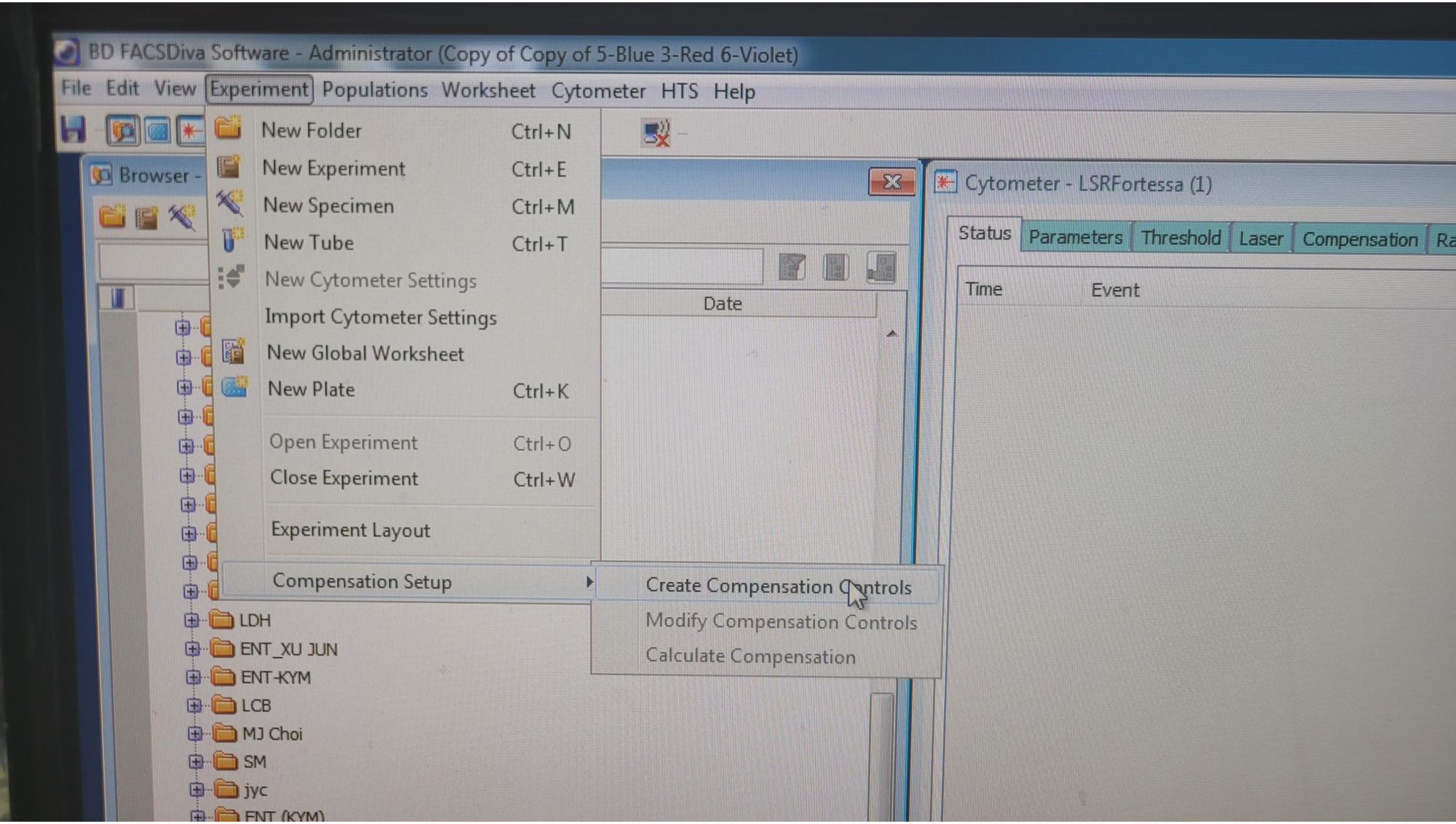


Fluorochrome	- % Fluorochrome	Spectral Overlap
BV650	BV605	0.00
FITC	BV605	0.00
APC-Cy7	BV605	0.00
BV605	BV650	0.00
FITC	BV650	0.00
APC-Cy7	BV650	0.00
BV605	FITC	0.00
BV650	FITC	0.00
APC-Cy7	FITC	0.00
BV605	APC-Cy7	0.00
BV650	APC-Cy7	0.00
FITC	APC-Cy7	0.00

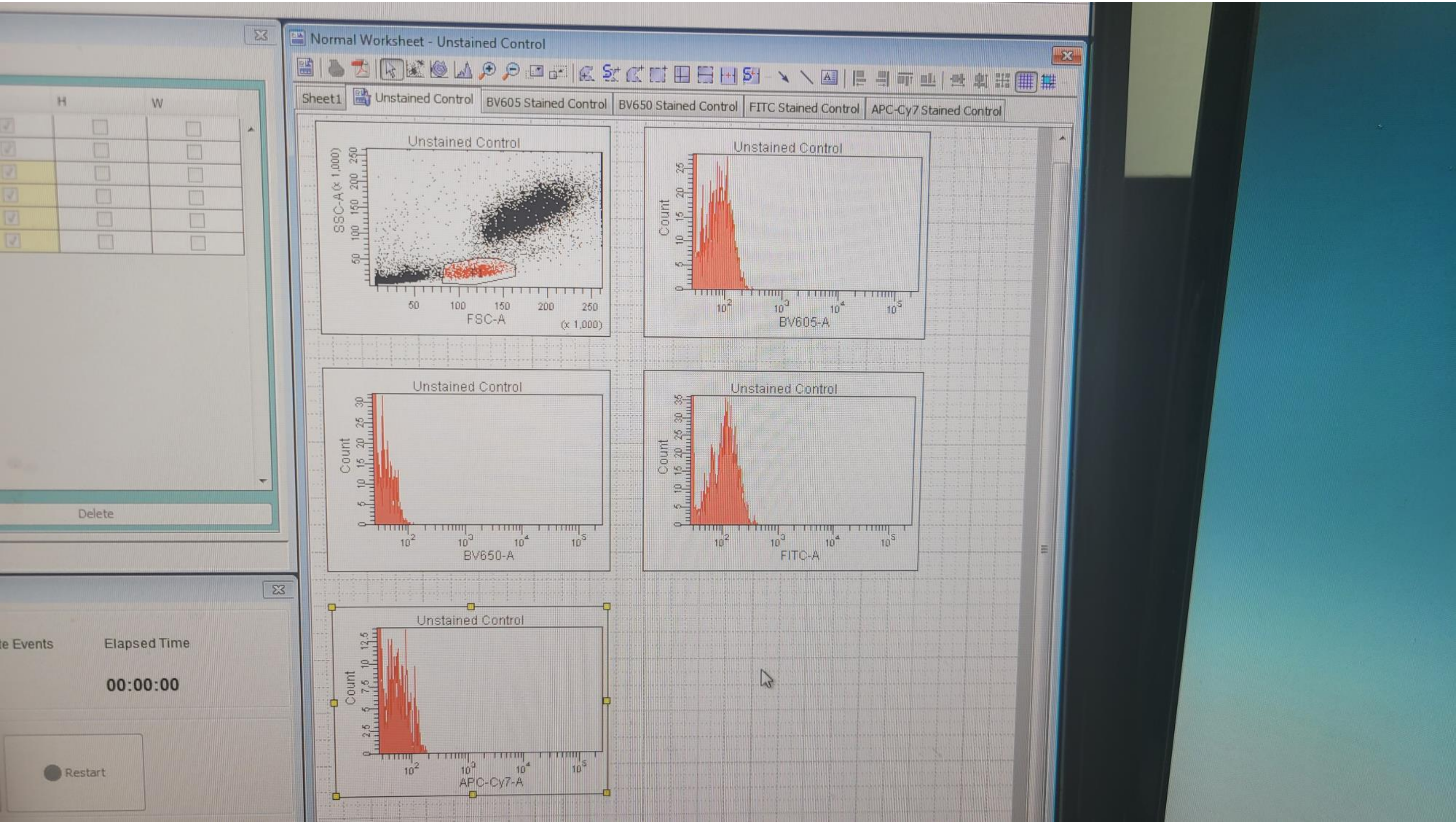
Compensation



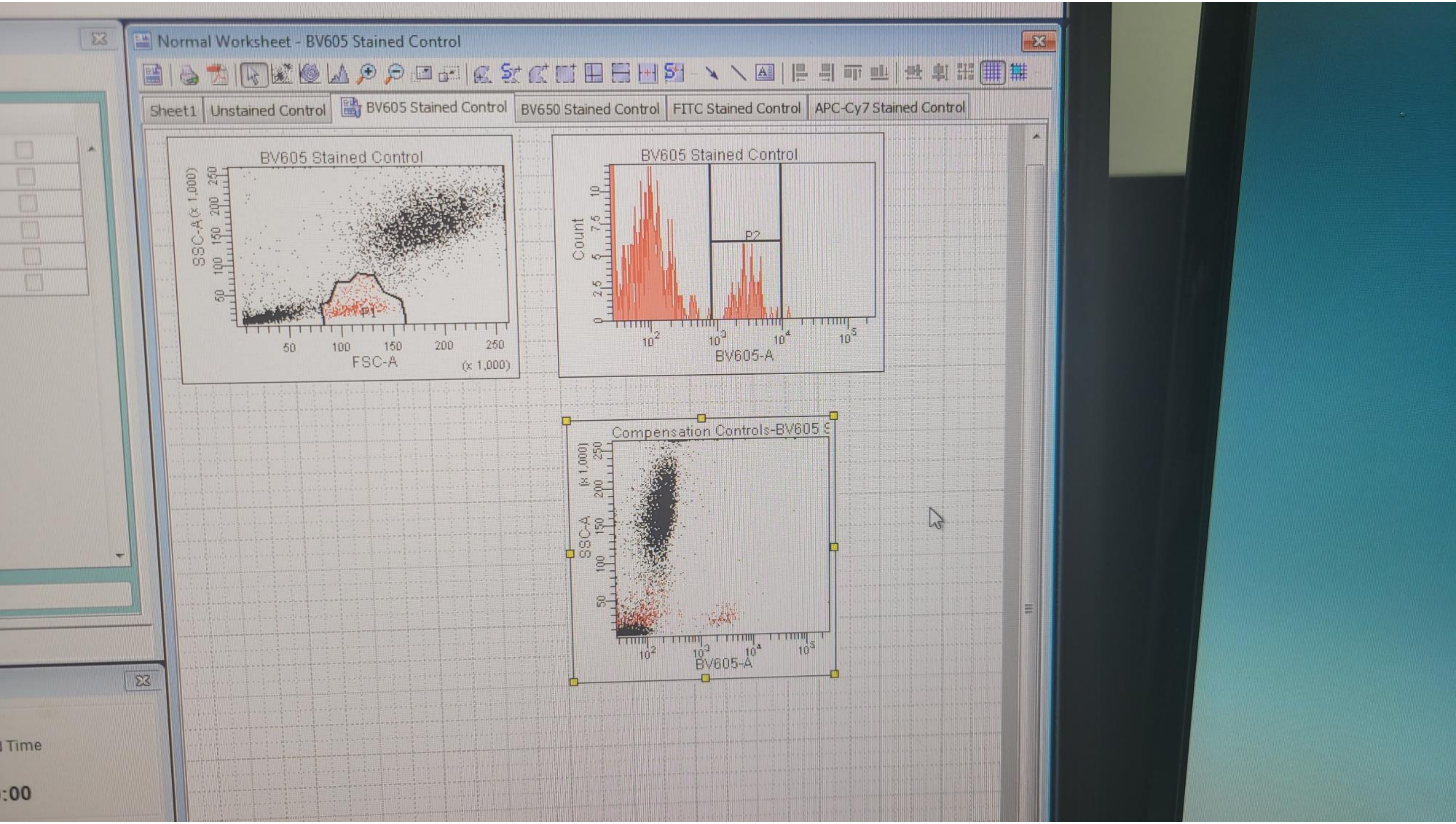
Compensation



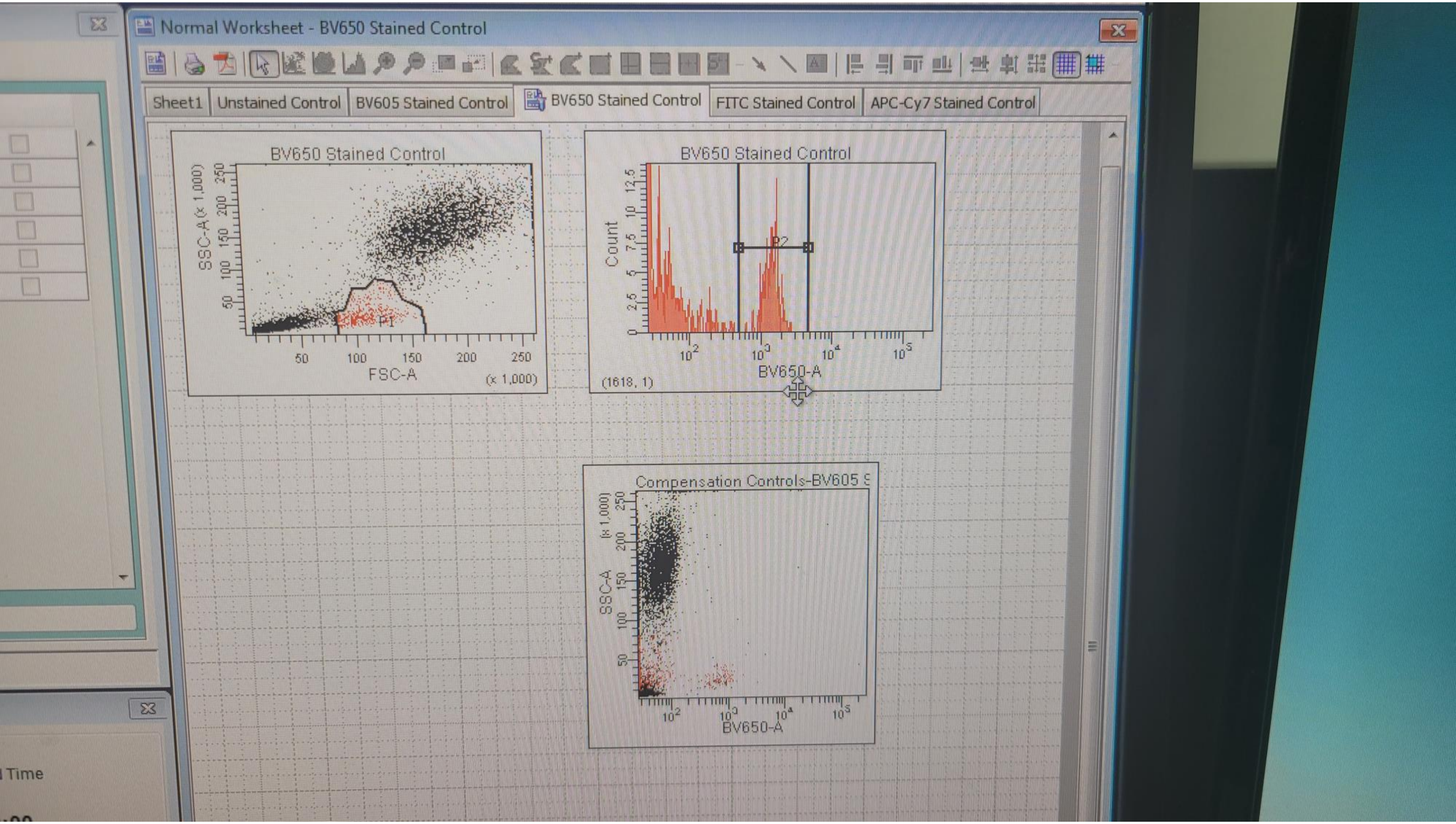
Compensation



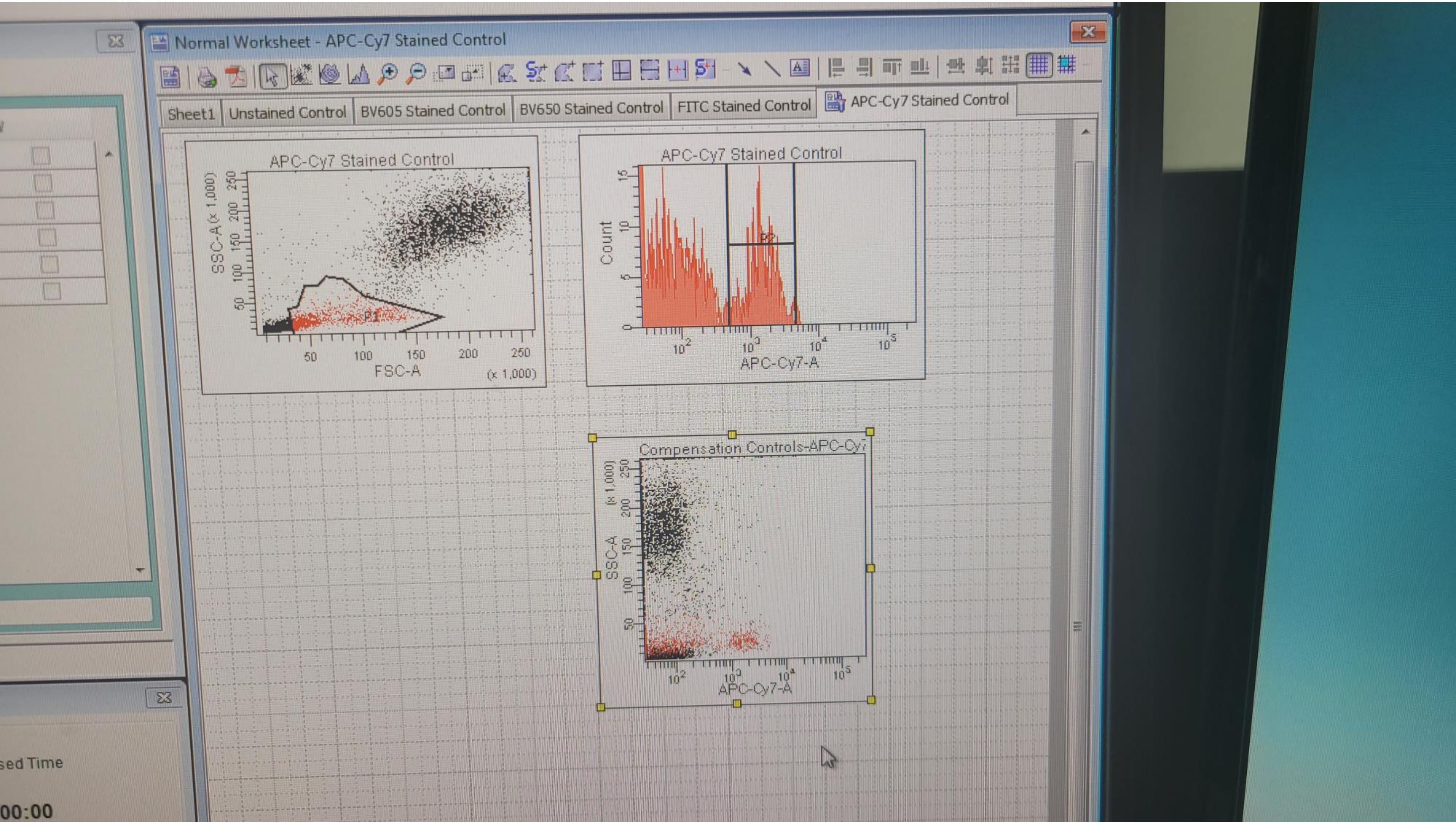
Compensation



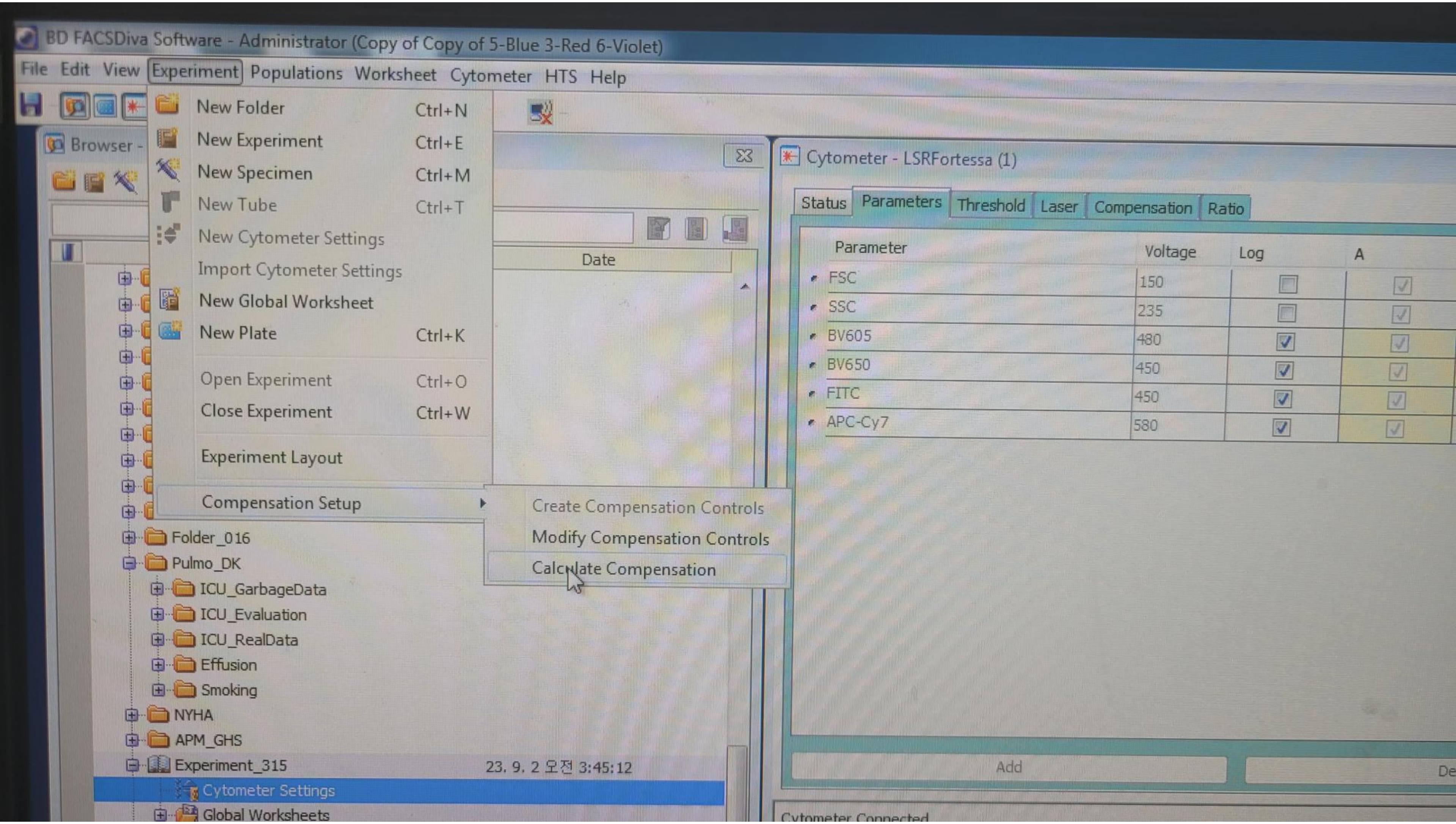
Compensation



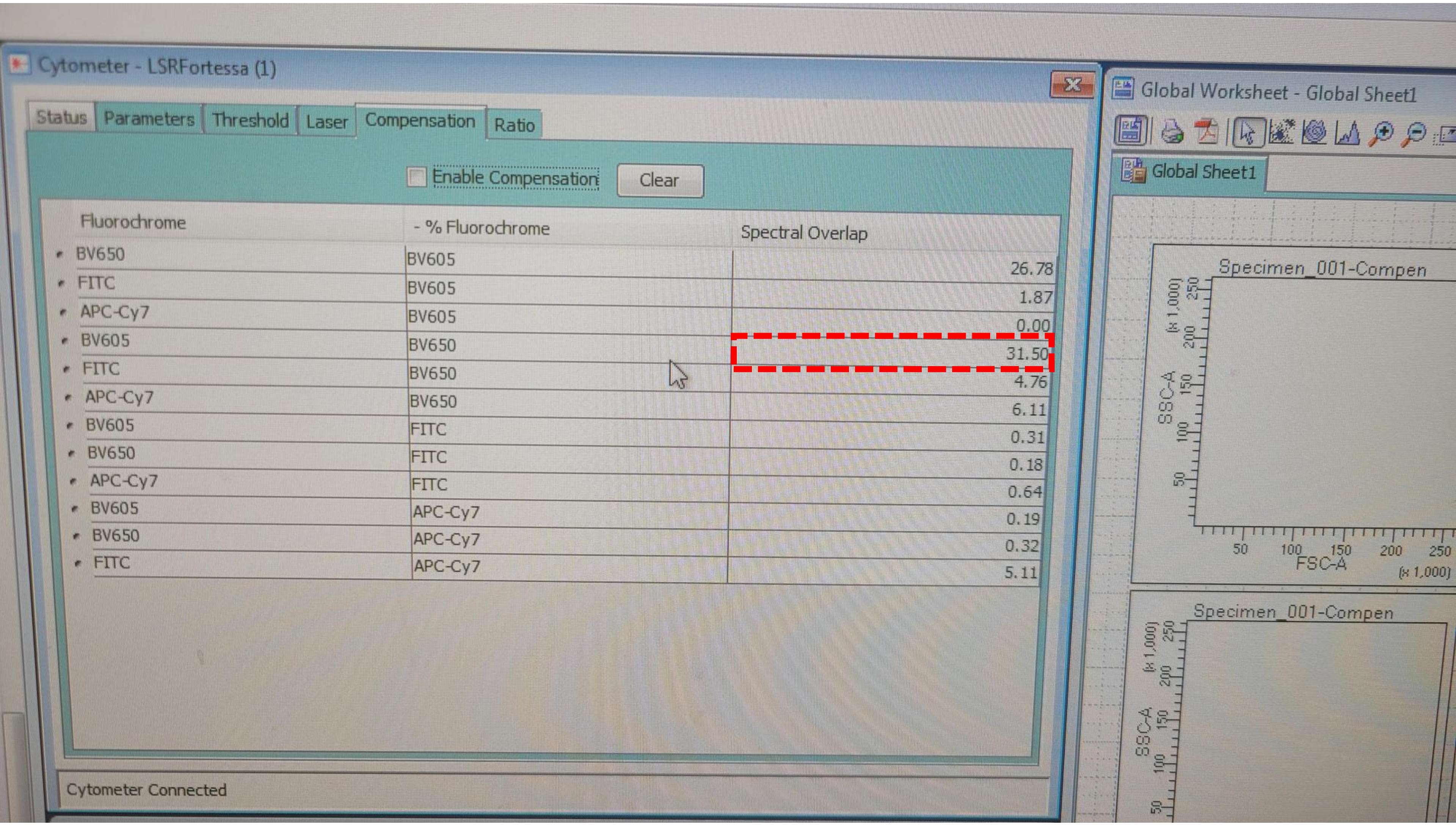
Compensation



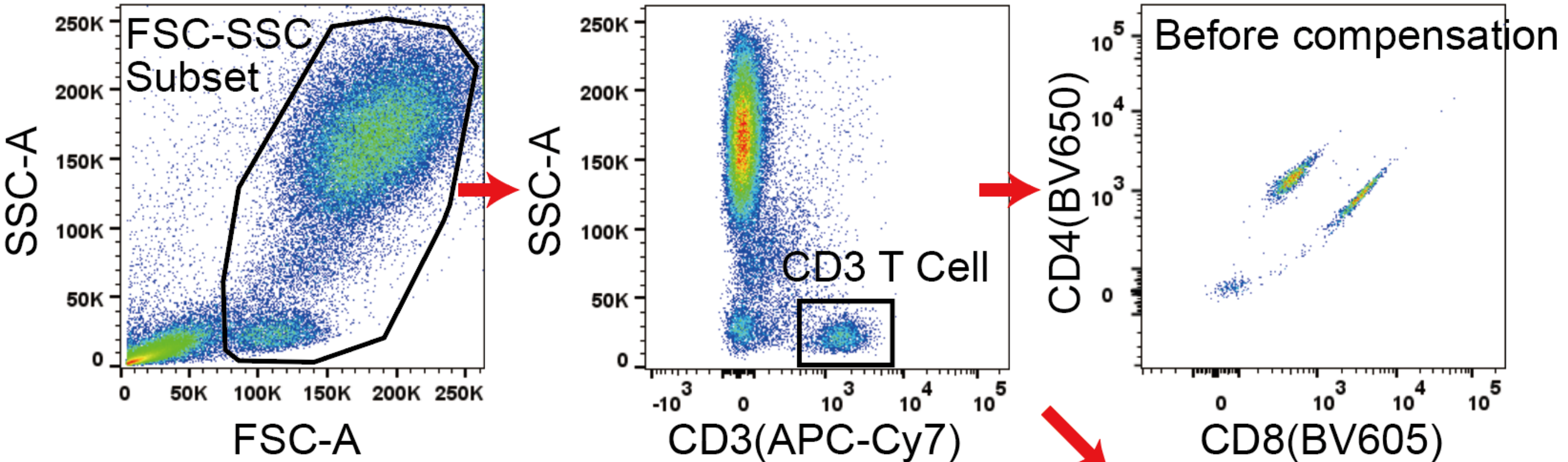
Compensation

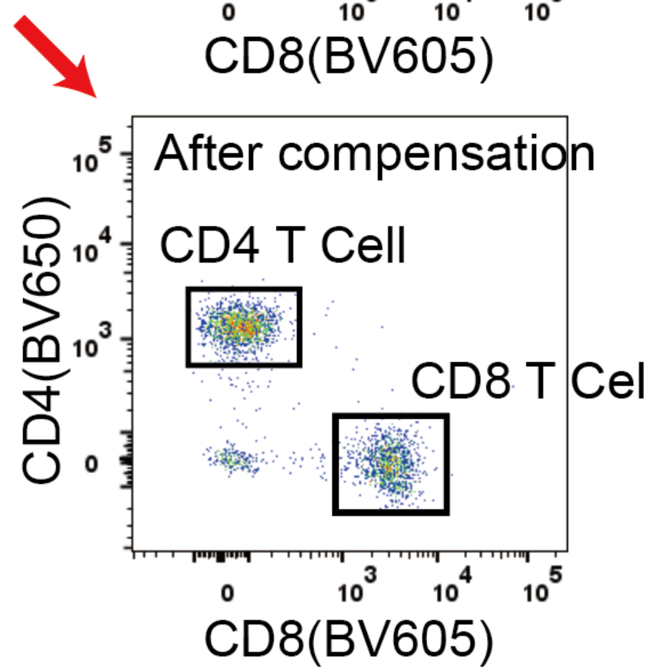


Compensation



Compensation

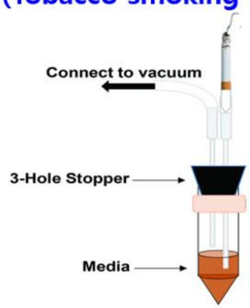




"올바른 **compensation**을 통해
각 형광사이의 **spill over** 된 값을 보정하여야만
정확한 데이터를 얻을 수 있다."

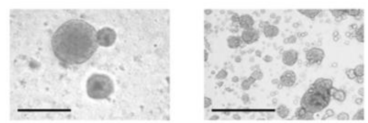
담배 추출물을 활용한 실험예시

담배 추출물 노출
(Tobacco smoking extract)



control, 0.25%, 0.5%, 1% 흡연 추출물 노출

폐 오가노이드 변화 분석



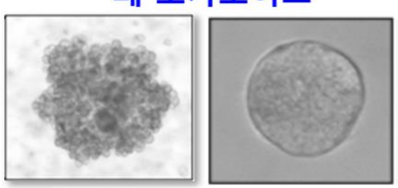
No. of organoids
Organoid growth rate
Size of organoids

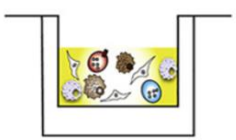
충남대학교병원
정재욱 교수님

흡연과 관련된 폐암 오가노이드 모델 구축

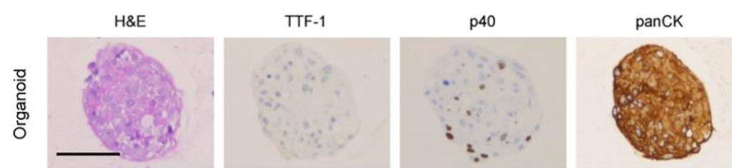
폐암 특이적 바이오 마커 발굴

폐 오가노이드



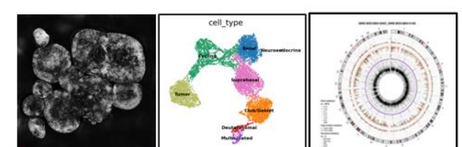


공동 배양 기술 개발

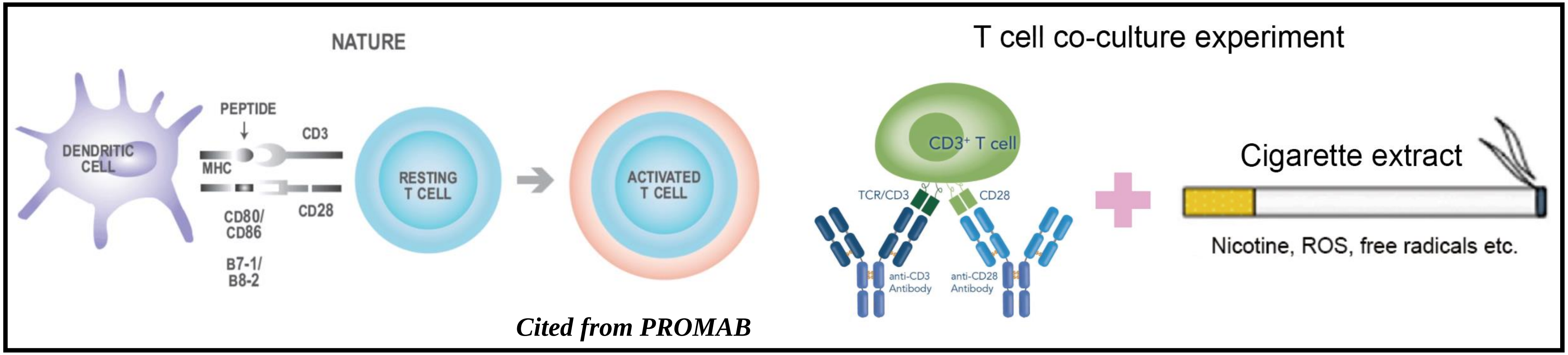


폐/폐암 오가노이드의 구조 및 단백질/유전자 발현 분석

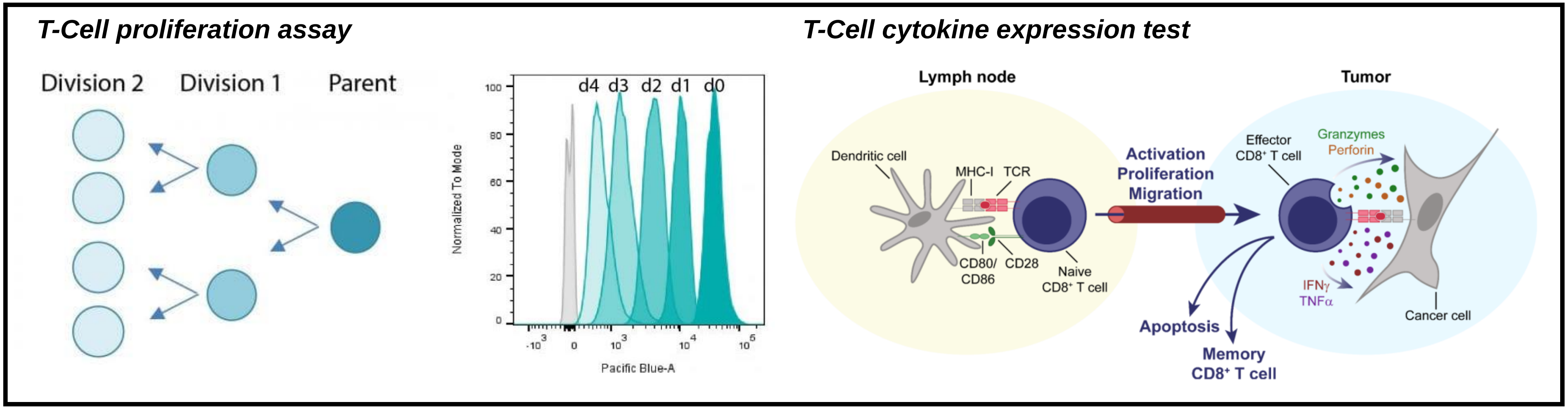
- H & E, Western Blot, 면역염색, Microarray 분석,
  3D 이미징, 단세포 RNA 분석, NGS 검사를 바탕으로
  흡연 추출에 노출된 폐 오가노이드의 유전자 및 단백질 발현 확인



담배 추출물을 활용한 실험예시

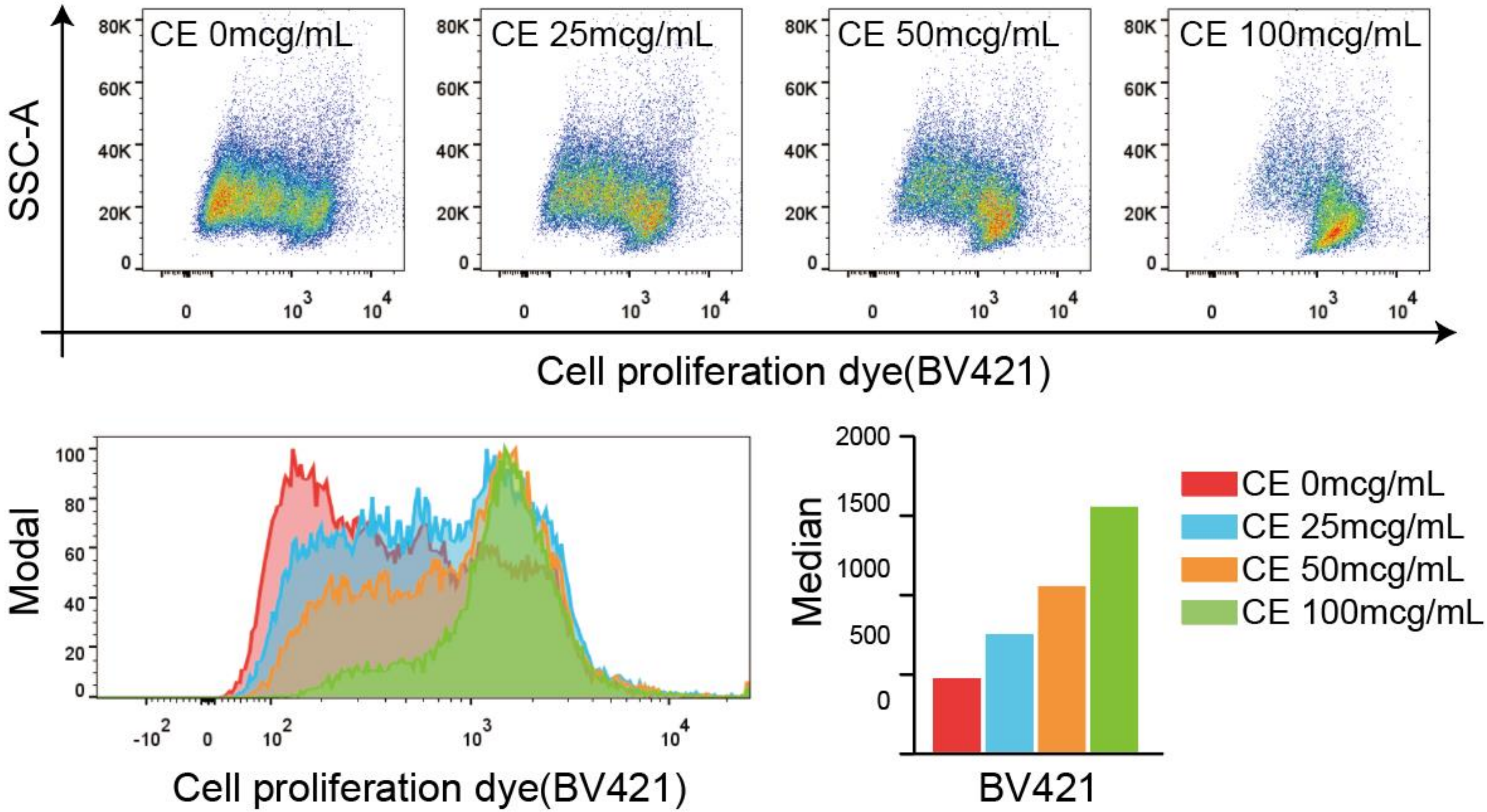


Cited from PROMAB



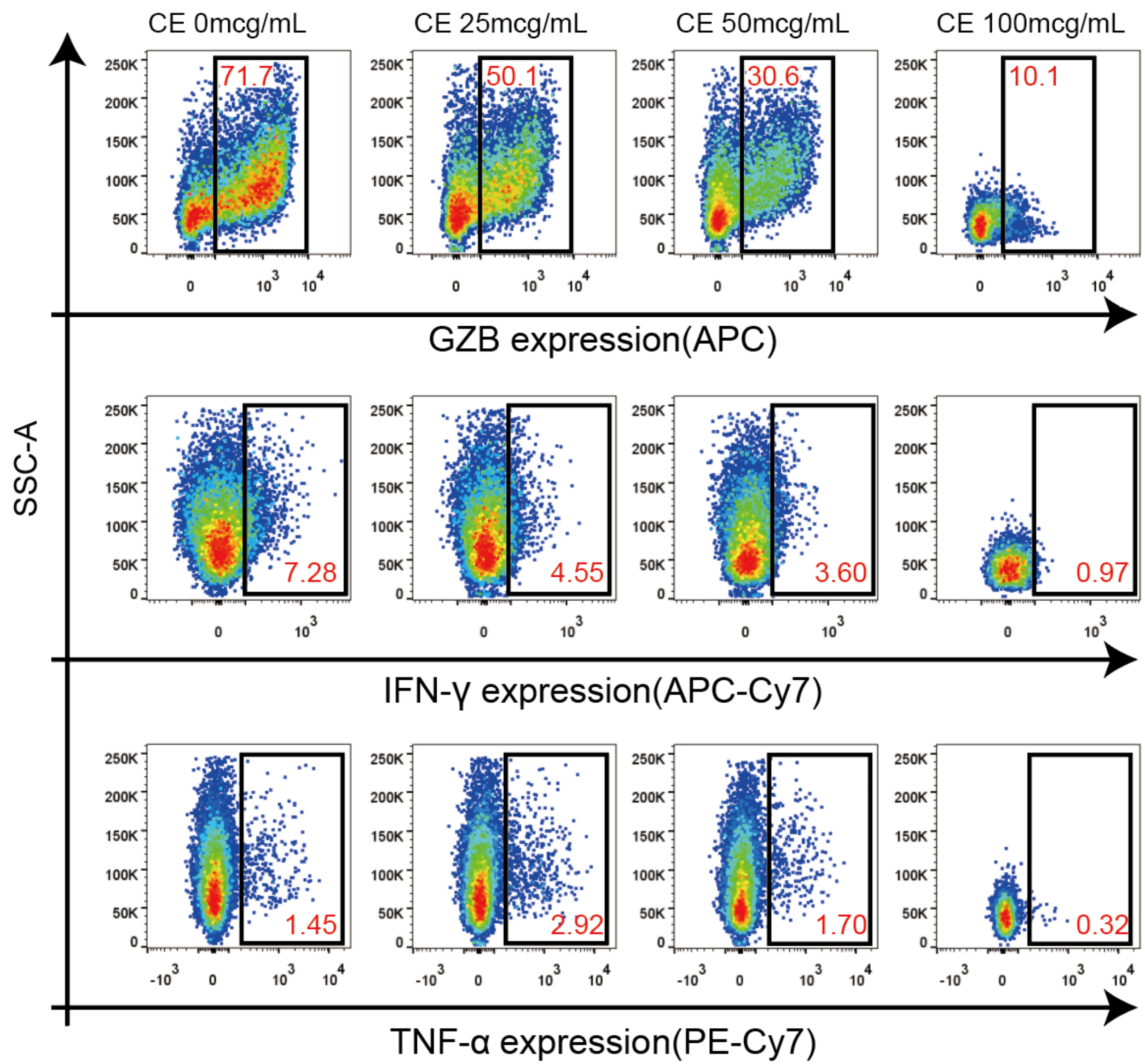
담배 추출물을 활용한 실험예시 – T cell proliferation assay

**CE : Cigratte extract



담배 추출물을 활용한 실험예시 – T cell cytokine expression test

**CE : Cigratte extract



Acknowledgement

Prof. Dong-il Park ***Prof. Chaeuk Chung***

Thank you for listening

Department Respiratory and Critical Medicine in CNUH