INSTITUTO DE MEDICINA MOLECULAR JOÃO LOBO ANTUNES

TRAINING CONTENT FOR ANALYSERS

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Accuri C6 Plus (1h30):

- Switch on the instrument;
- Maintenance and quality control (CST);
- Login Computer;
- Accuri C6 Plus Software
- Setting up an Experiment:
    - BD Accuri C6 Plus workspace (collect, analyse, statistics and batch analysis),
    - Acquiring data,
    - Creating graphics (histograms, dot plots or density),
    - Setting limits for the acquisition,
    - Controlling the fluidics + Backflush + SIP clean,
    - Defining the gates and obtain statistics,
    - Explaining what are compensations (automatic ones with the CST or manual) and how to perform;
- Save and export the data; creating templates; Save PowerPoint and Excel files;
- Data deletion;
- Cytometer cleaning (<10evts/s when acquiring water);
- Fill the sheath fluid and discard the waste;
- Sample preparation – Dilution, concentration and filtering;
- Pipetes, tubes, gloves, solutions, etc;
- Log sheet and Incidents Report (Agendo);
- Booking system;
- Explain training process with assistance, when independent will change do Regular Usage;
- Request assistance;
- Unit rules (working hours, assistance schedule, booking cancelations, turning ON and OFF the systems, contact other users).

Fortessa (2h):

- Switch on the instrument;
- Maintenance and quality control;
- Fortessa: FFSS, computer and cytometer;
- Troubleshooting (clog > PRIME and clean, FACSDiva is not connected > log off computer / switch of computer and cytometer, no events > check the tube / connection issue (restart DIVA) /Emergency contact)
- Login Computer;
- Software BD FACSDiva (deliver the password),
- Setting up an Experiment:
    - FACSDiva windows (Browser, Cytometer, Inspector, Worksheet and Acquisition Dashboard),
    - Create a new experiment and specimen,
    - Select parameters,
    - Explain FSC and SSC,
    - Creating a Dot Plot and Histogram,
    - Creating a Gate,
    - Selecting Parameter for Dot Plot Axes,
    - Setting Up Voltages Based on Unstained/Negative Control,
    - Adjusting Area Scaling for FSC;
    - Checking the Single Color Tubes;
    - Recording Data (number of events to record, Stopping Gate, storage gate);
    - Compensation Manually and Automatically;
    - Global Worksheet/Normal Worksheet.
- Acquire samples (PBS between samples);
- Explain doublet exclusion;
- Labels;
- Draw graphs, gates, populations Hierarchy;
- How to duplicate an experiment without data;
- Export Data (Experiment, FCS Files, Template);
- Data Deletion;
- Cytometer cleaning (Record);
- Fill the sheath fluid and discard the waste;
- Sample preparation – Dilution, concentration and filtering;

- Pipetes, tubes, gloves, solutions, etc;
- Log sheet and Incidents Report (Agendo);
- Booking system;
- Explain training process with assistance, when independent will change to Regular User;
- Request assistance;
- Unit rules (working hours, assistance schedule, booking cancelations)
- Turning ON and OFF the systems, contact other users.

HTS (30min):

- Change from tube mode to HTS mode (Explain the importance of selecting *Run* and never *Standby*);
- Explain the reinitialize and priming modes and how to eliminate bubbles in the system;
- Software DIVA applied to plate reading;
- Acquisition: High throughput (22 uL only) vs standard;
- Loader settings (Explain all the parameters; Pay attention to sample volume and mixing volume; Explain dead volume (20 uL); adjust the number of mixes depending on the cells we are running – increase if they are sticky);
- Plate compensations (can be done in tube mode, and then change for the HTS to run the samples or in the plate directly);
- Sample preparation – Dilution, concentration and filtering;
- Maintenance and quality control;
- Save and export the data, creating templates, Data deletion;
- HTS cleaning;
- Log sheet.

Symphony (2h):

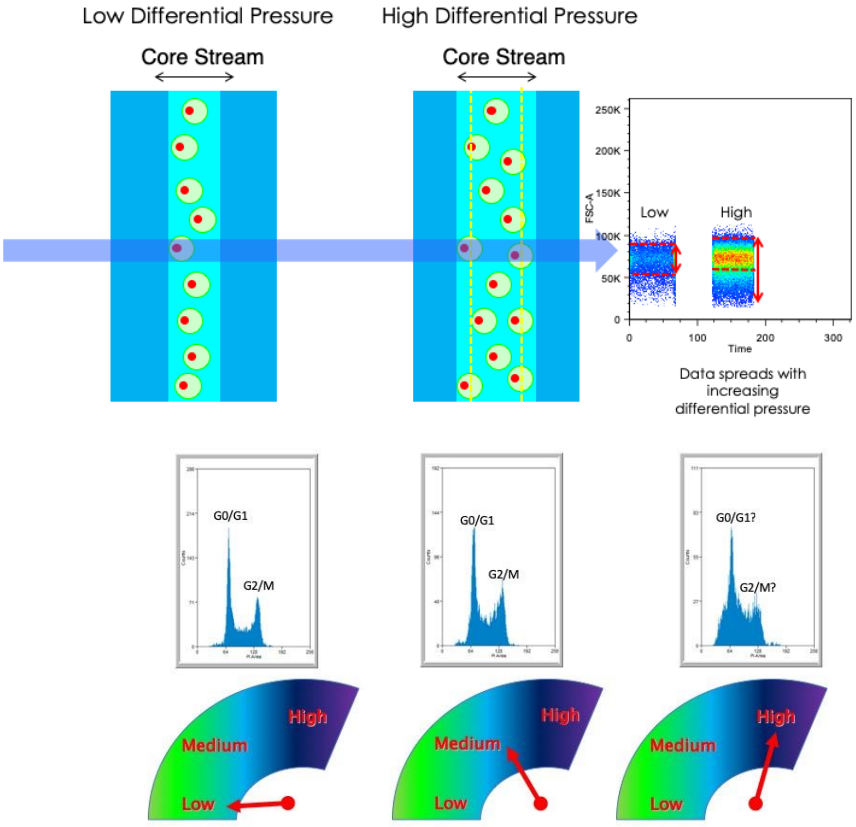
- Switch on the instrument (FFSS, computer and cytometer);
- Login Computer;
- Switch on lasers;
- Software BD FACSDiva (deliver the password or create a new one),
- Cleaning and prime check;
- Quality Control (Run a performance check with correct vial – with warnings > proceed; error > call the emergency number);
- Troubleshooting (clog > clean (avoid PRIME), FACSDiva is not connected > log off computer / switch of computer and cytometer, no events > check the tube / connection issue (restart DIVA) /Emergency contact)
- Setting up an Spectral Experiment:
    - FACSDiva windows (Browser, Cytometer, Inspector, Worksheet and Acquisition Dashboard)
    - Create a new experiment and specimen
    - Explain FSC and SSC and adjust
    - Adjust Area Scaling for FSC
    - Creating a spectral plot
    - Explain AutoF
    - Explain Standardization
    - Check if everything is on scale (multicolor tube)
    - Checking the Autofluorescence channel in the unstained cells Tube
    - Checking the Single Color Tubes (primary channel)
    - Experiment layout
    - Create a spectral unmixing matrix
    - Record compensation controls (Reinforce the importance of creating a P3 negative population if looking at different cell types from the Unstained-P1 or beads)
    - Save a unmixing matrix
    - Creating a Dot Plot and Histogram
    - Creating a Gate,
    - Selecting Parameters in the Dot Plot Axes,
    - Recording Data (number of events to record, Stopping Gate, storage gate);
    - Compensation and spectral unmixing and uncompensated (interchangeable);

- To apply a different compensation/unmixing: cytometer settings > apply catalog
- Global Worksheet/Normal Worksheet.

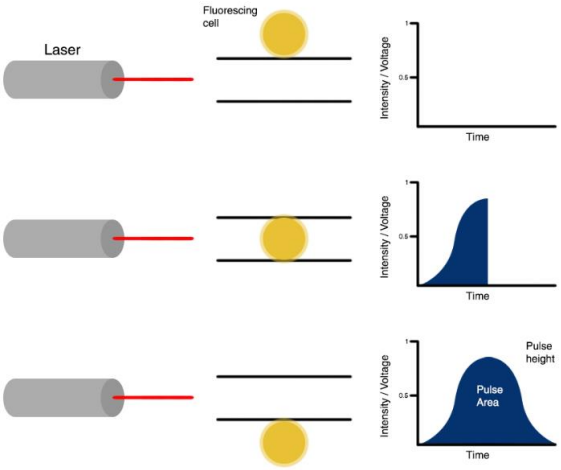
- Acquire samples (PBS between samples);
- Explain doublet exclusion;
- Draw graphs, gates, populations Hierarchy;
- Export:
  - Experiment (1st time): to re-do the unmixing and/or overwrite some single color;
  - Template (1st time): to reuse every day
    - Panel template (standard.) vs Experiment Template (non-standard.)
  - Data (Daily): Unmixed FCS files;
- Data Deletion;
- Cytometer cleaning (Record);
- Turn OFF Lasers
- Fill the sheath fluid tank and discard the waste tank;
- Sample preparation – Dilution, concentration and filtering;
- Pipetes, tubes, gloves, solutions, etc;
- Log sheet and Incidents Report (Agendo);
- Booking system;
- Explain training process with assistance, when independent will change to Regular User;
- Request assistance;
- Unit rules (working hours, assistance schedule, booking cancelations)
- Turning ON and OFF the systems, contact other users.

Appendix A

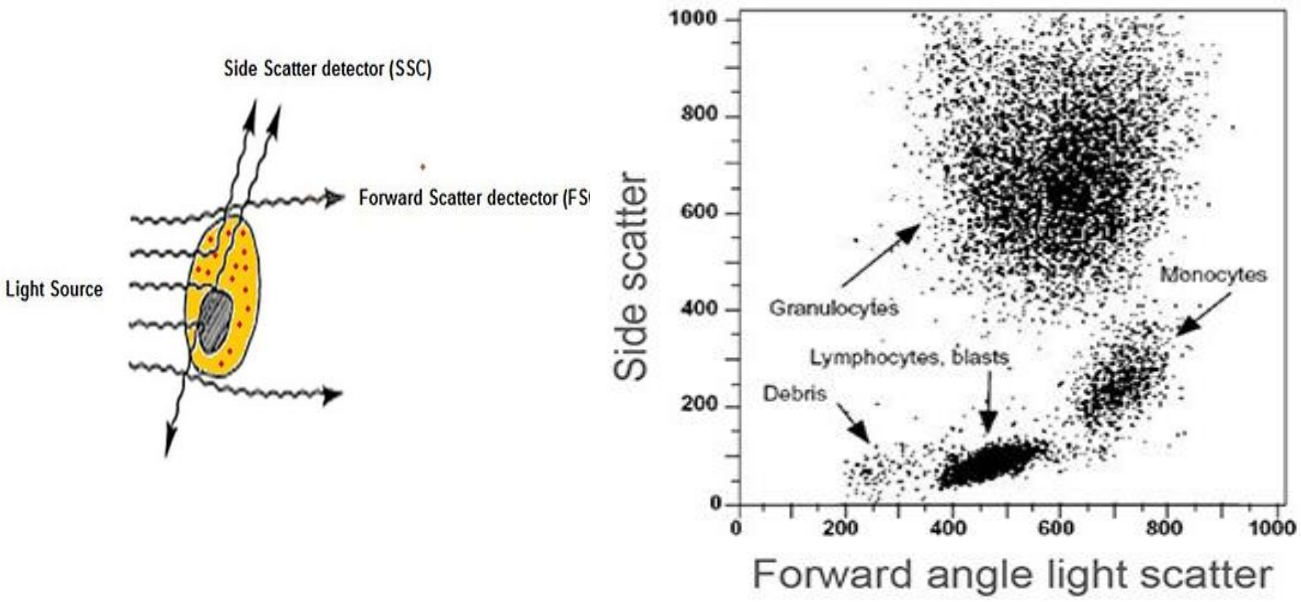
FLUIDICS



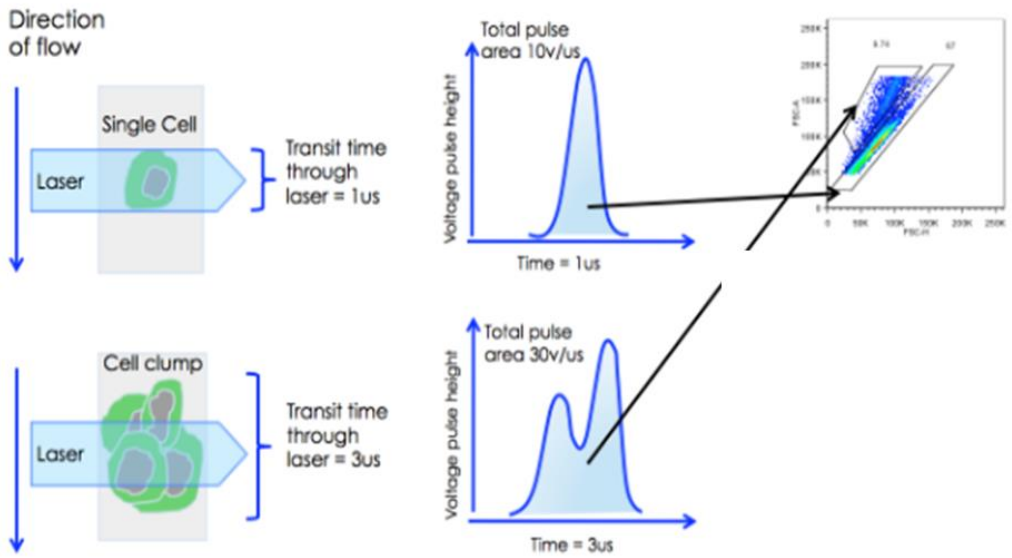
ELECTRONICS



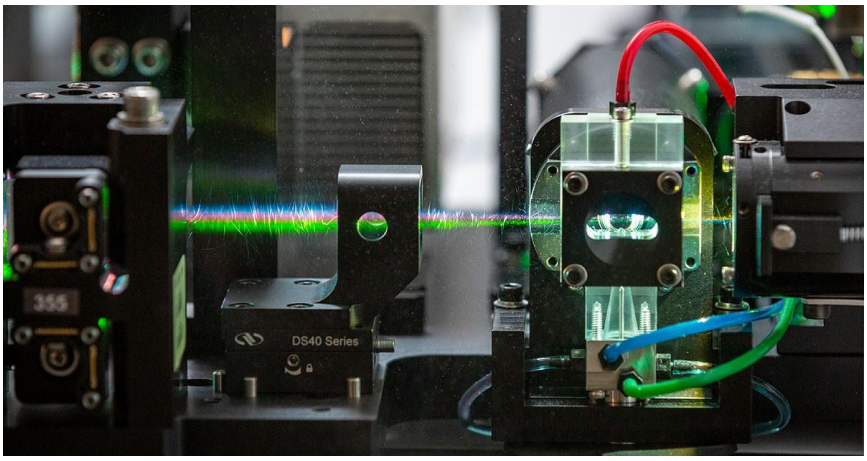
FSC AND SSC PARAMETERS

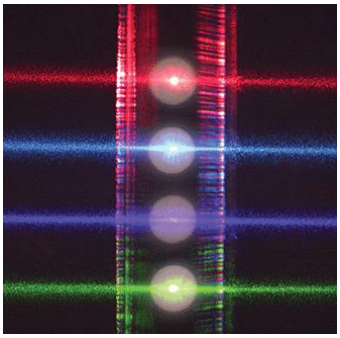


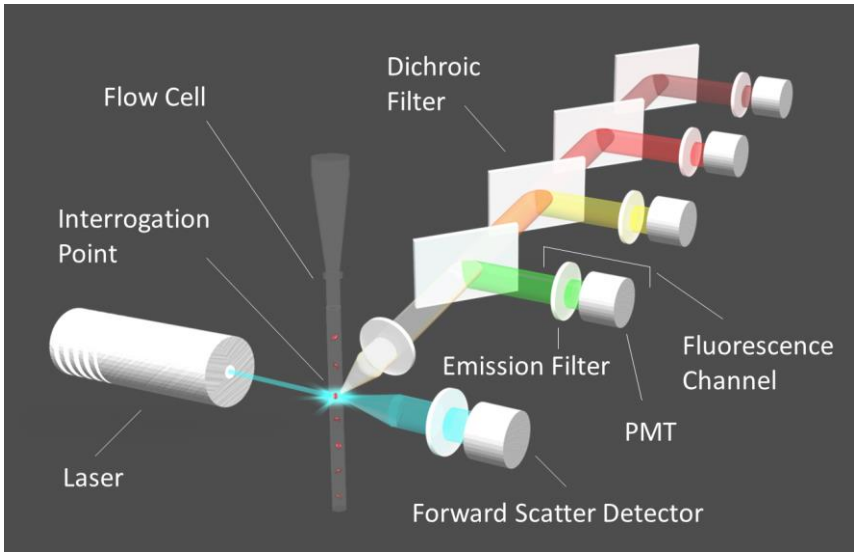
DOUBLET DISCRIMINATION



OPTICS OF A CONVENTIONAL FLOW CYTOMETER







CONVENTIONAL VS SPECTRAL FLOW CYTOMETER

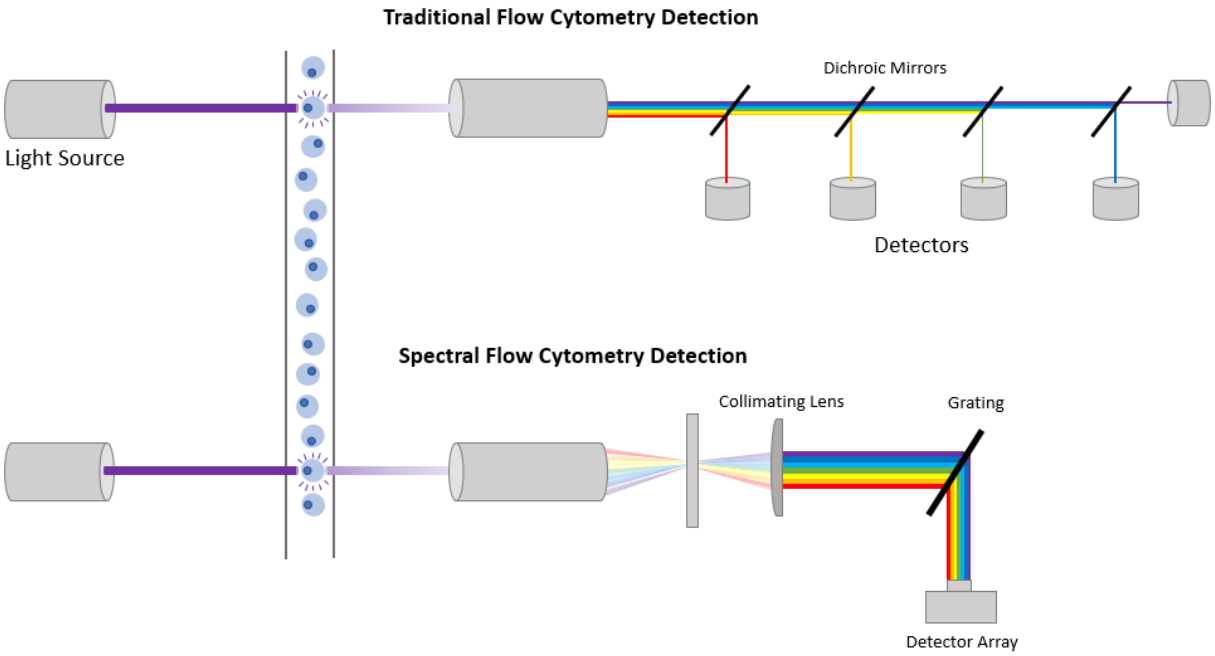
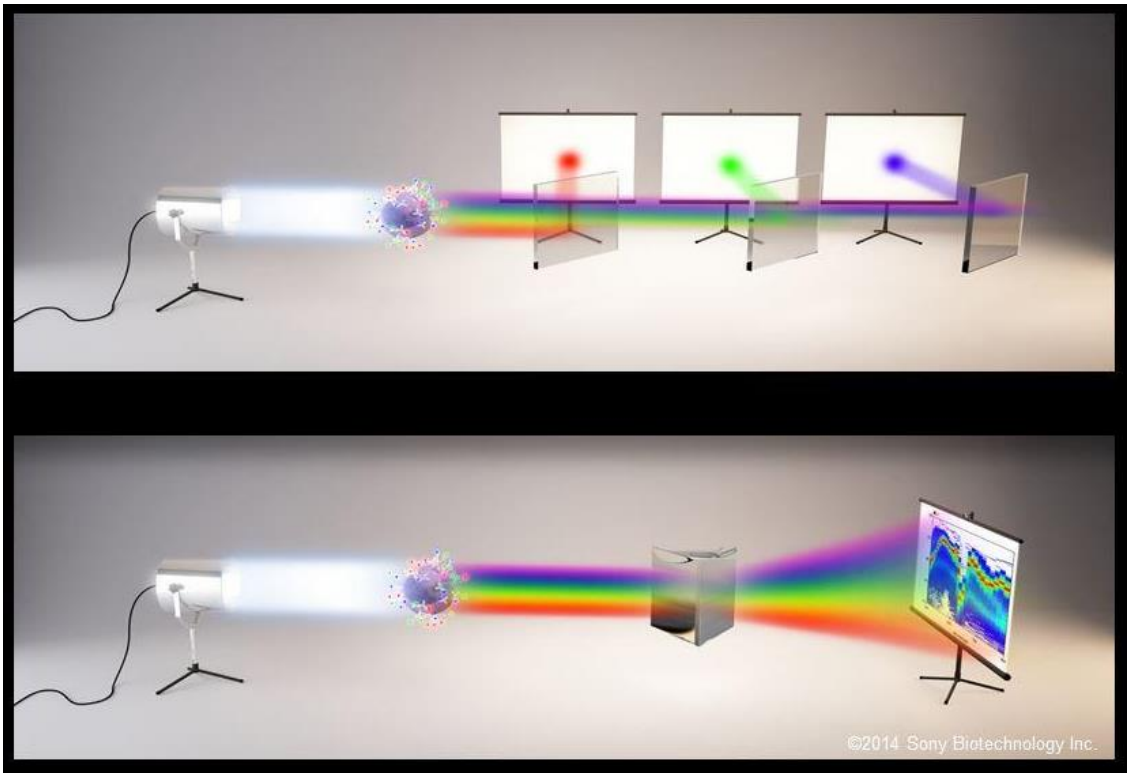
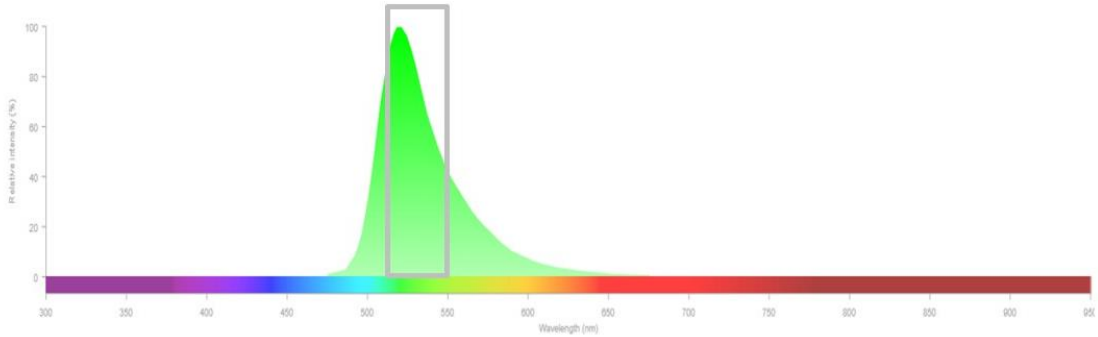


Figure 1. Comparison of traditional and spectral flow cytometry detection mechanisms.



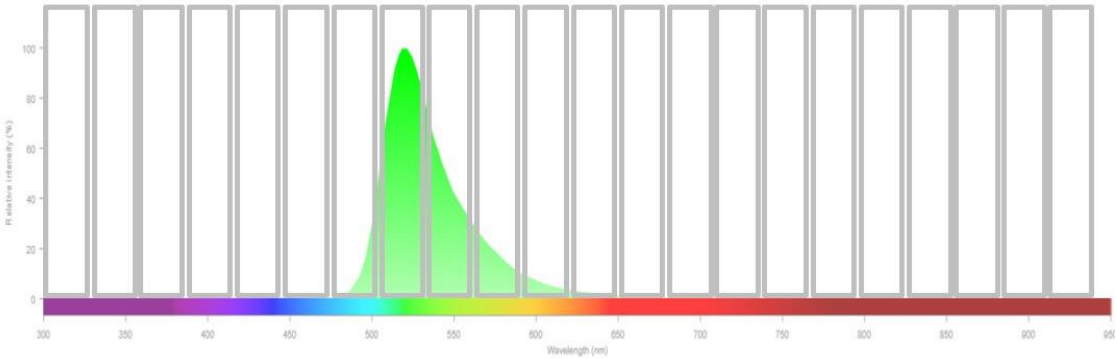
EMISSION SPECTRUM
(Conventional Flow Cytometry)

A.



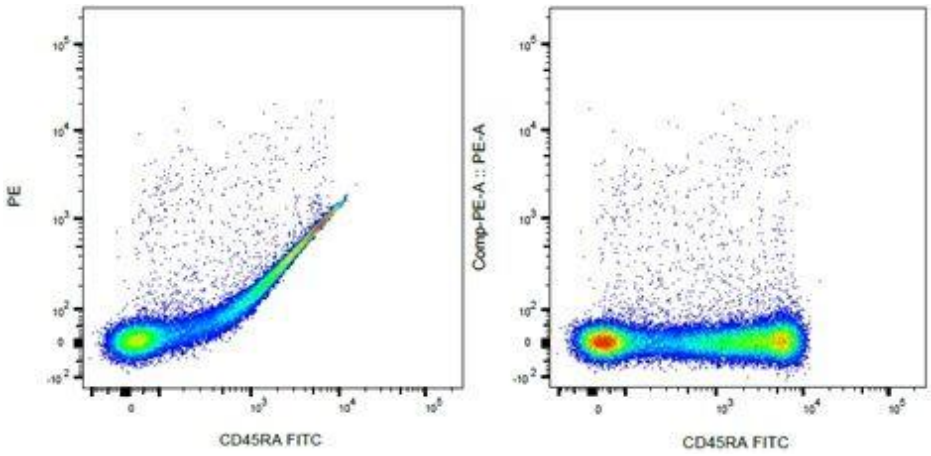
EMISSION SPECTRUM
(Spectral Flow Cytometry)

B.



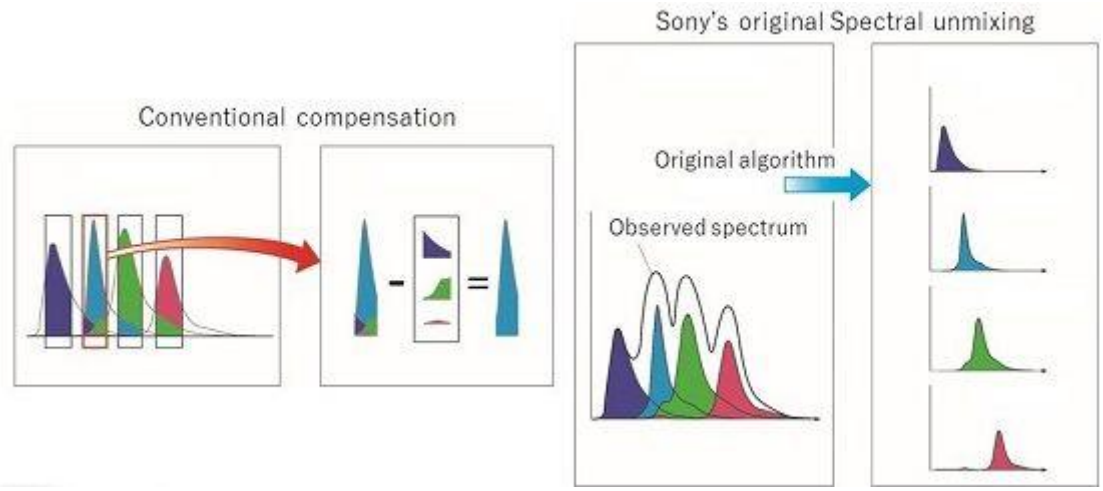
COMPENSATION

FITC SINGLE COLOR



UNCOMPENSATED COMPENSATED

COMPENSATION VS SPECTRAL UNMIXING



GRAPHICS AND GATING