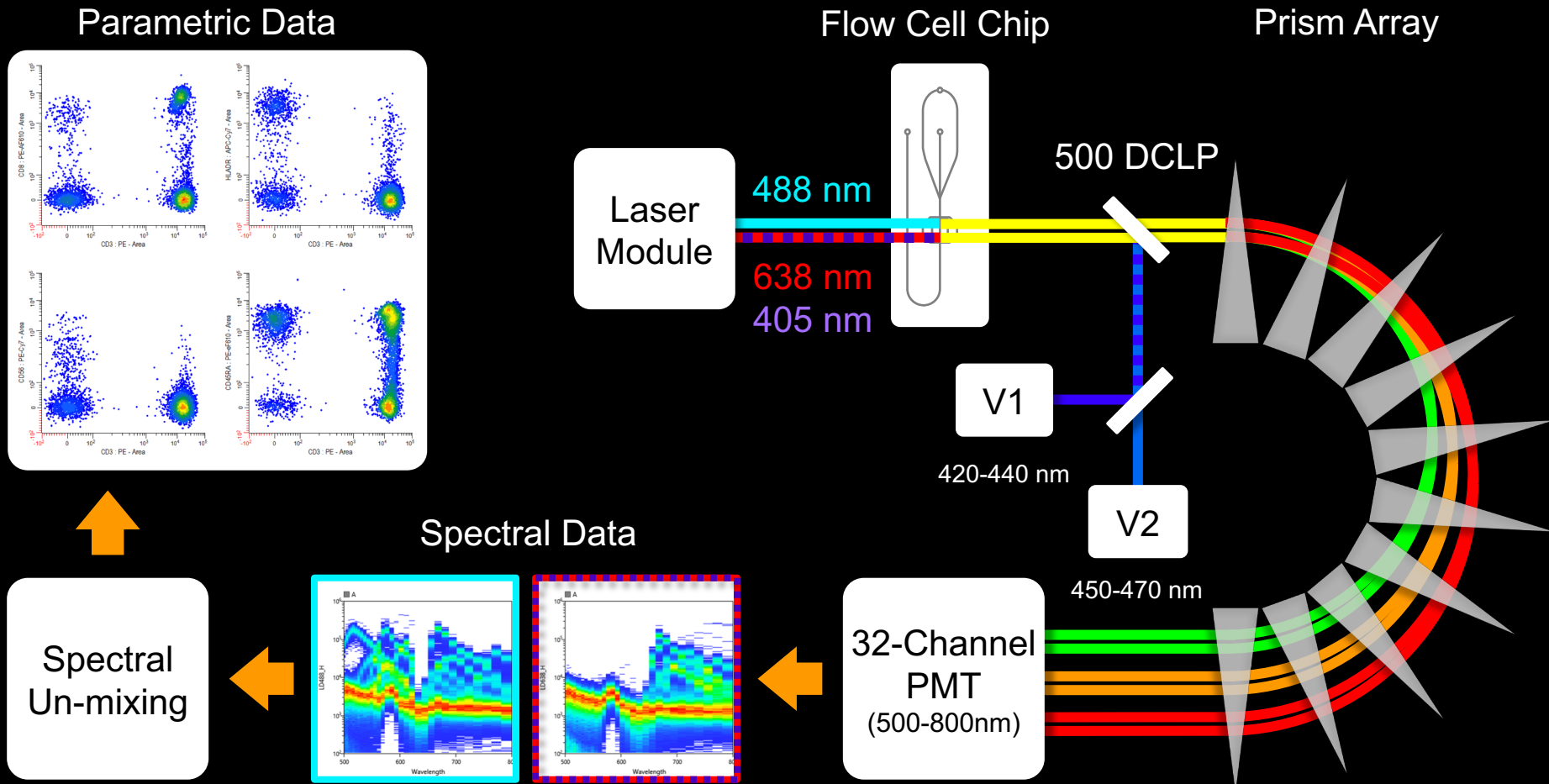




SP6800
Spectral Analyzer

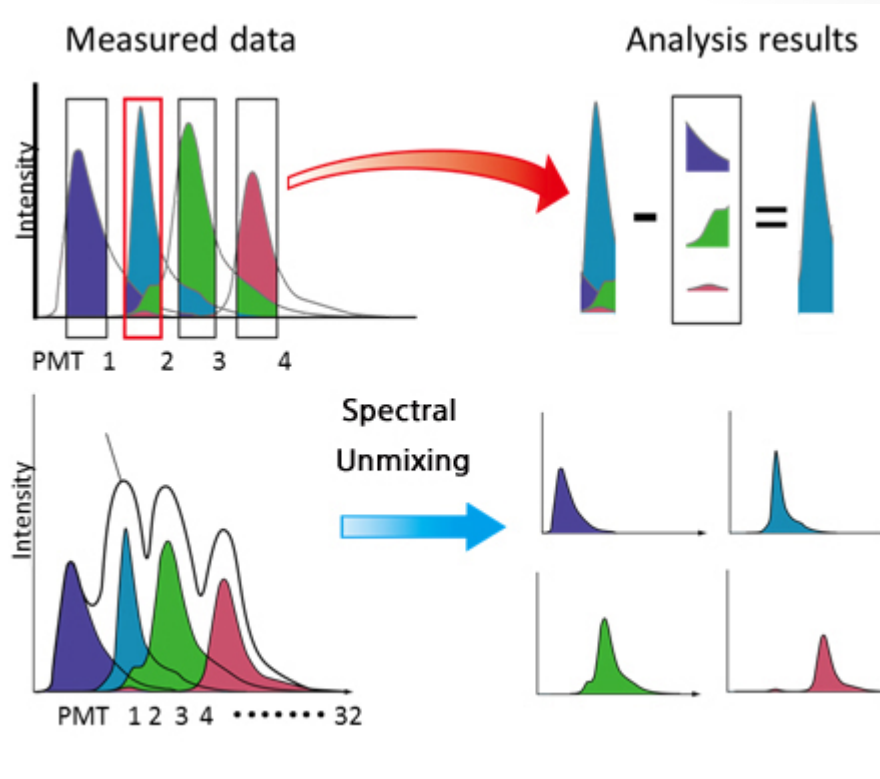
SP6800 Optical Path



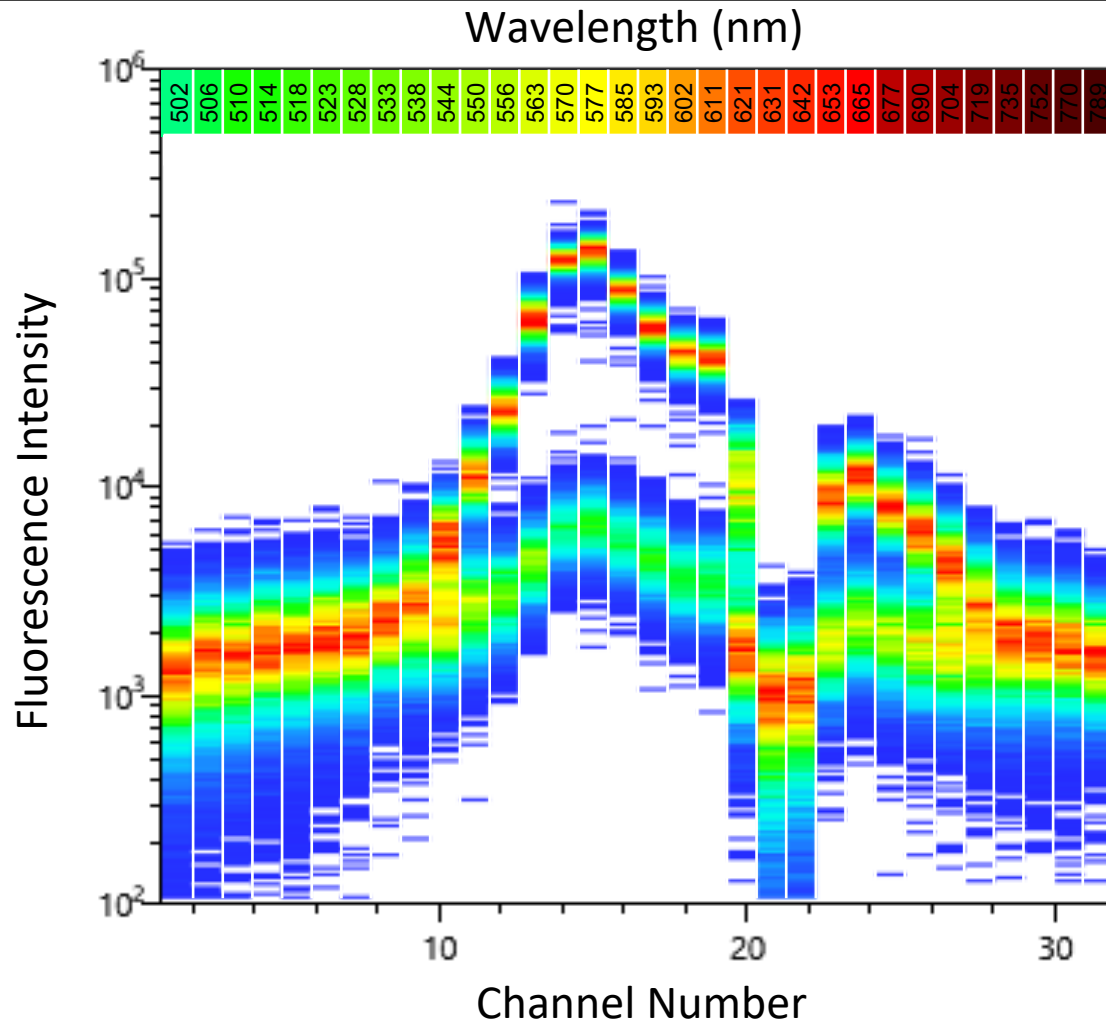
Conventional & Spectral Flow cytometry

Conventional FACS:
Bandpass filters & detectors
Algorithm for spectral overlap compensation

Spectral flow analyzer:
One detector for whole spectrum
Algorithm for unmixing of individual fluorescence emission

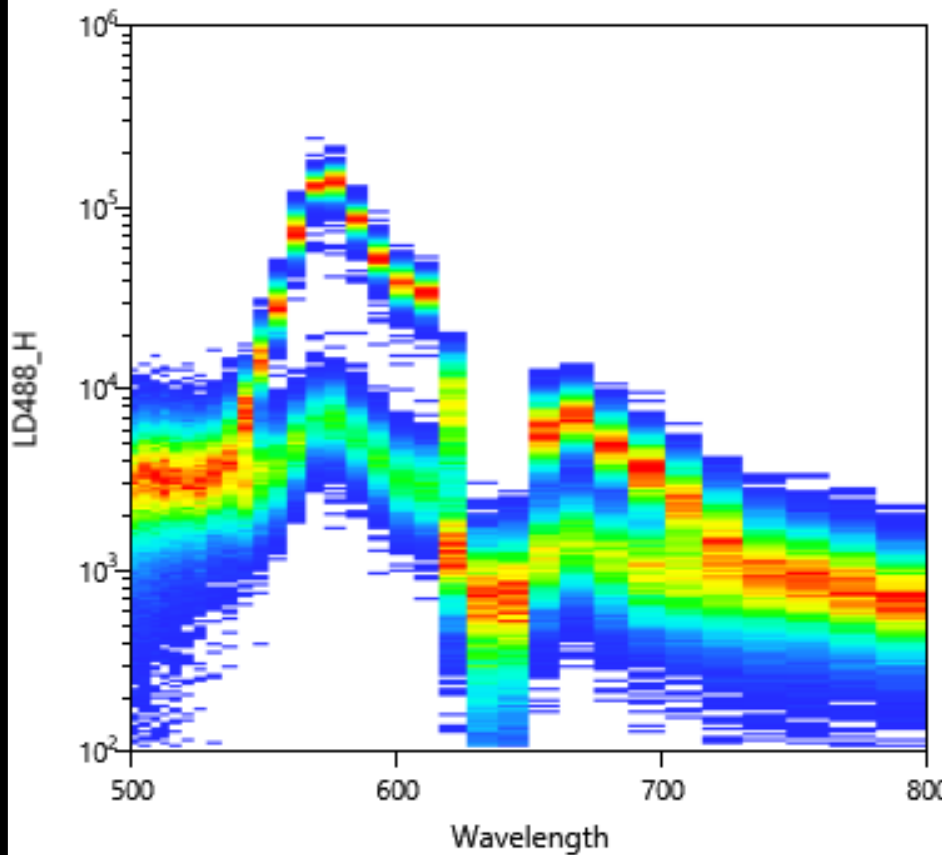


Spectral data plot

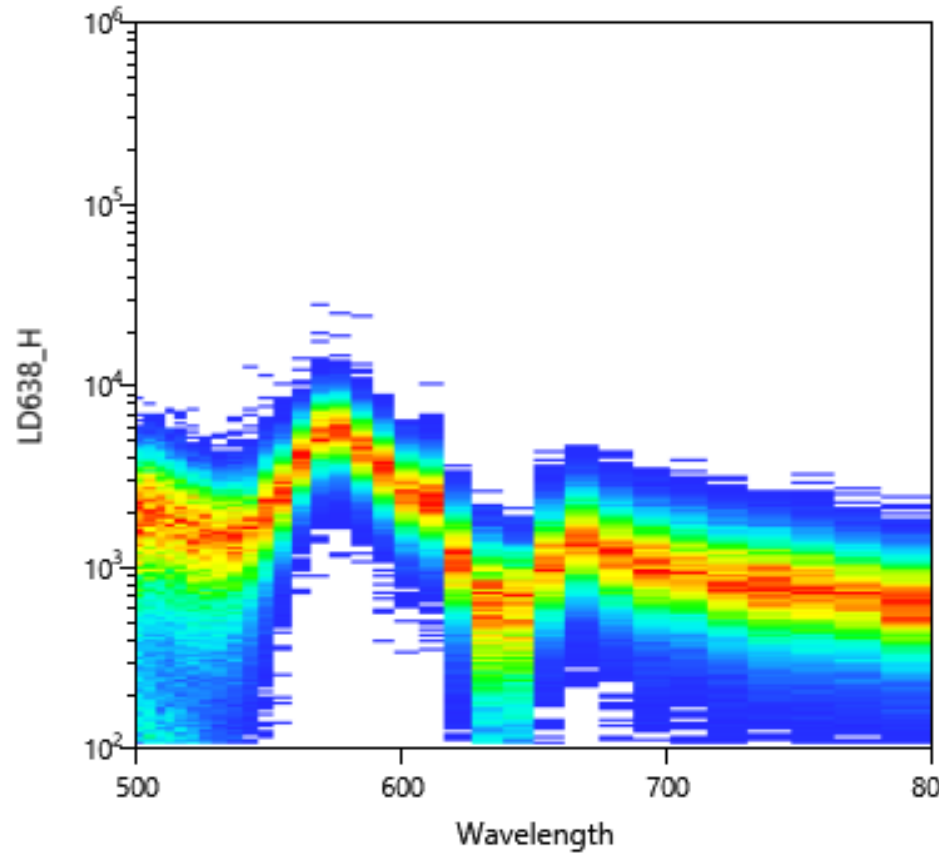


Spectral Data from two laser intercepts

Top Laser (Blue)



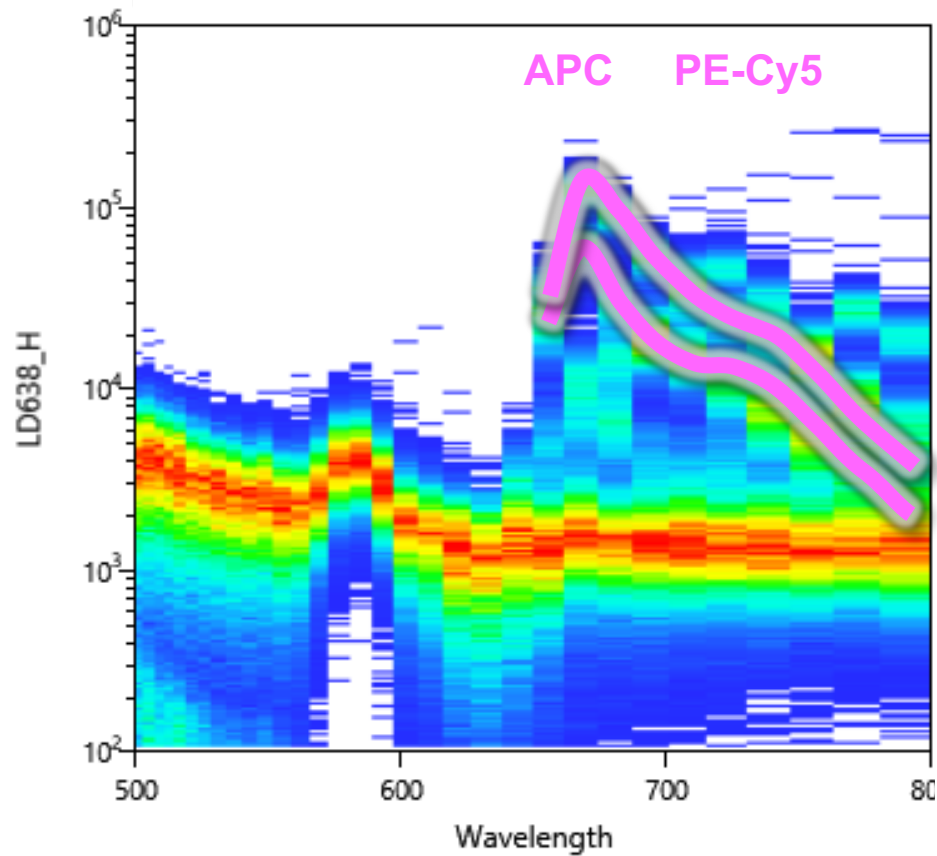
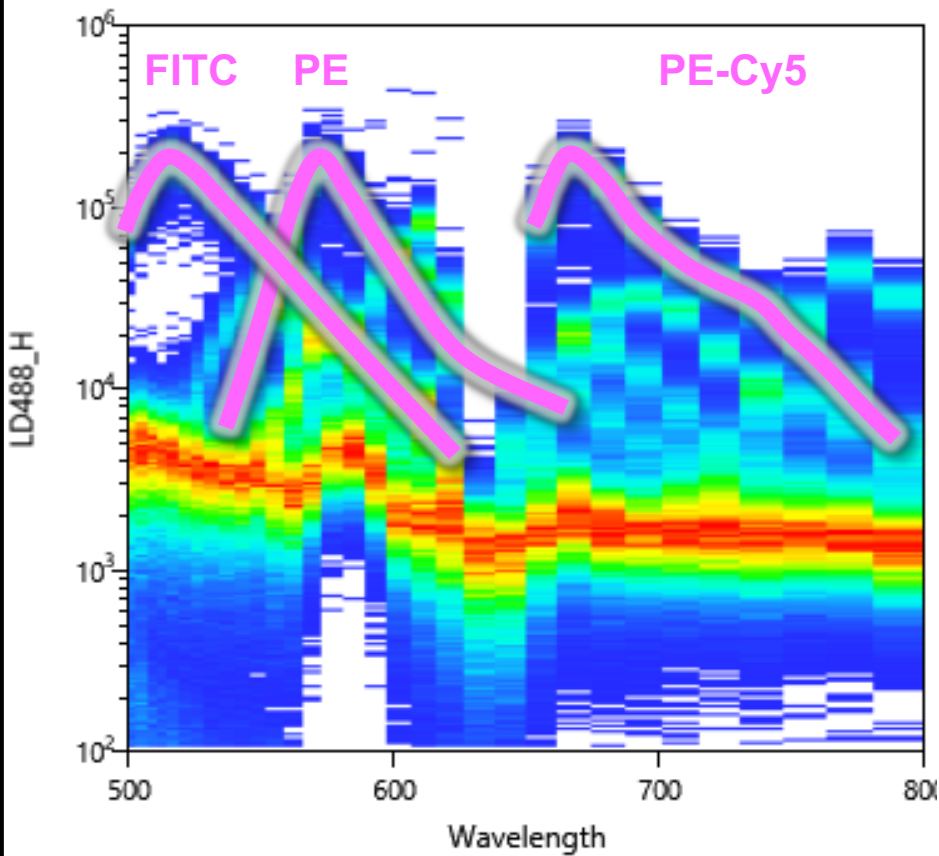
Bottom Laser (Red)



Full spectrum data: Mix of single-stained beads

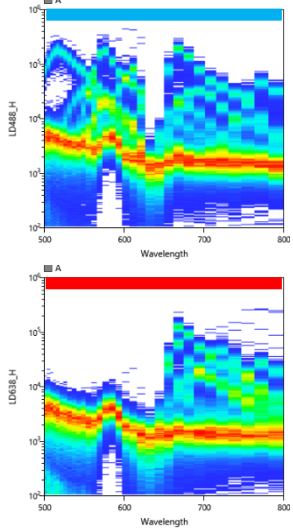
Blue Laser

Red Laser

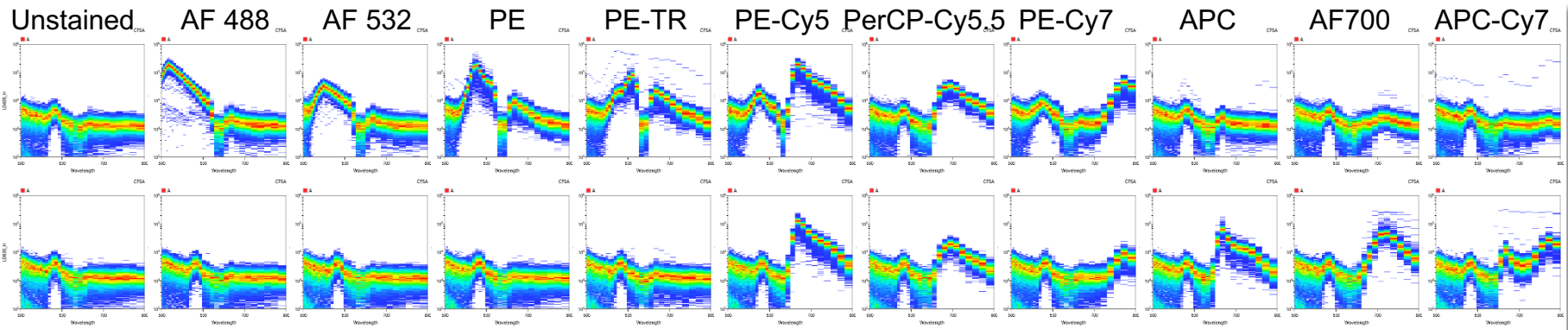


Workflow for spectral analysis

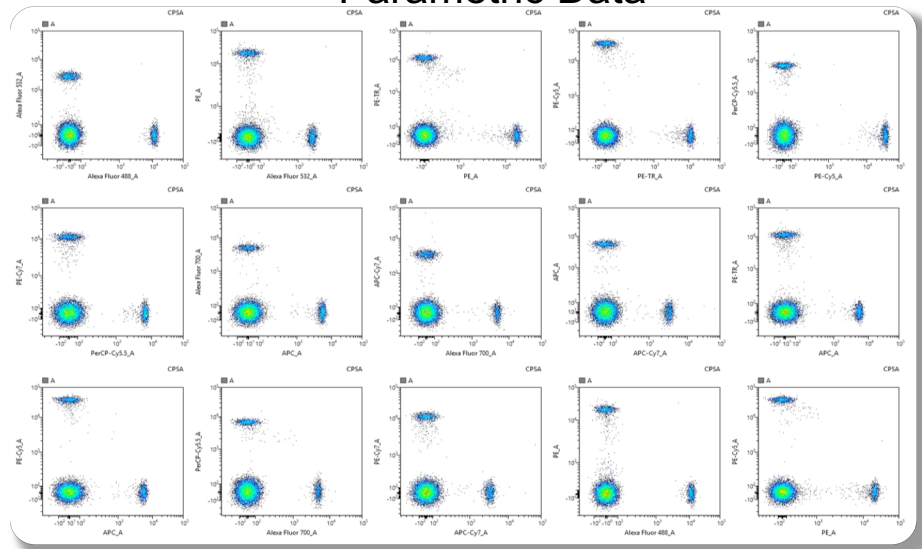
Spectral Data



Reference Spectra



Parametric Data



SONY SP6800 Spectral Analyzer



Kenmerken Sony SP6800:

- 3 lasers, waarvan 2 collinair (488, 405/638nm)
- 32 kanalen per PMT detector



Sony SP6800 Software: acquisition mode

Control
Status: Ready

Acquisition
Total Event: 0
Acquisition Time: 00:00:00
Elapsed Time: 00:00:00
Event Rate: 0 eps
Saturation Rate: 0.0 %

Stopping Condition
Total Events: 10,000

Tube List

| Name | Events |
|-------------------|--------------|
| Unst | 5,475 |
| fitc | 3,680 |
| PE | 10,000 |
| PE-CF594 | 10,000 |
| PE-Cy5.5 | 10,000 |
| PE-Cy7 | 10,000 |
| BV421 | 2,569 |
| BV510 | 10,000 |
| BV605 | 1,264 |
| BV650 | 10,000 |
| APC | 5,251 |
| Alexa Fluor 700 | 2,195 |
| APC-Cy7 | 4,204 |
| all stains | 3,284 |
| FMO cd127 | 10,000 |
| FMO cd117 | 16,986 |
| FMO CRTH2 | 10,000 |
| FMO cd69 | 10,000 |
| all fmo's | 3,658 |
| Tube - 1 | |

Marker

| Marker | Fluorochrome | 488nm Laser | 405/638nm Laser |
|---------|--------------|-------------|-----------------|
| liv/cd3 | FITC | | |
| cd94 | PE | | |
| CRTH2 | PE-CF594 | | |
| cd117 | PE-Cy5.5 | | |
| cd127 | PE-Cy7 | | |
| cd200r | BV421 | | |
| cd161 | BV510 | | |
| cd45 | BV605 | | |
| NKp46 | BV650 | | |
| NKp44 | APC | | |
| cd69 | APC-Cy7 | | |
| cd3 | APC-Cy7 | | |

Unmixing
Unstained Sample: A (Unst) AF as Color
Universal Negative: A (Unst)

Fluorochrome

| Fluorochrome | Negative | Positive |
|-----------------|---------------------------------------|---|
| FITC | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> B (fitc) |
| PE | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> B (PE) |
| PE-CF594 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> B (PE-CF594) |
| PE-Cy5.5 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> B (PE-Cy5.5) |
| PE-Cy7 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> B (PE-Cy7) |
| BV421 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> C (BV421) |
| BV510 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> C (BV510) |
| BV605 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> C (BV605) |
| BV650 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> C (BV650) |
| APC | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> C (APC) |
| Alexa Fluor 700 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> C (Alexa Flu...) |
| APC-Cy7 | <input type="checkbox"/> (Univ. Neg.) | <input type="checkbox"/> C (APC-Cy7) |

Gates and Statistics

| Name | Events | Parent% | Total% |
|------------|--------|----------|----------|
| All events | 3,284 | 100.00 % | 100.00 % |
| A | 1,225 | 37.30 % | 37.30 % |

Main Plot Area (WLSM):

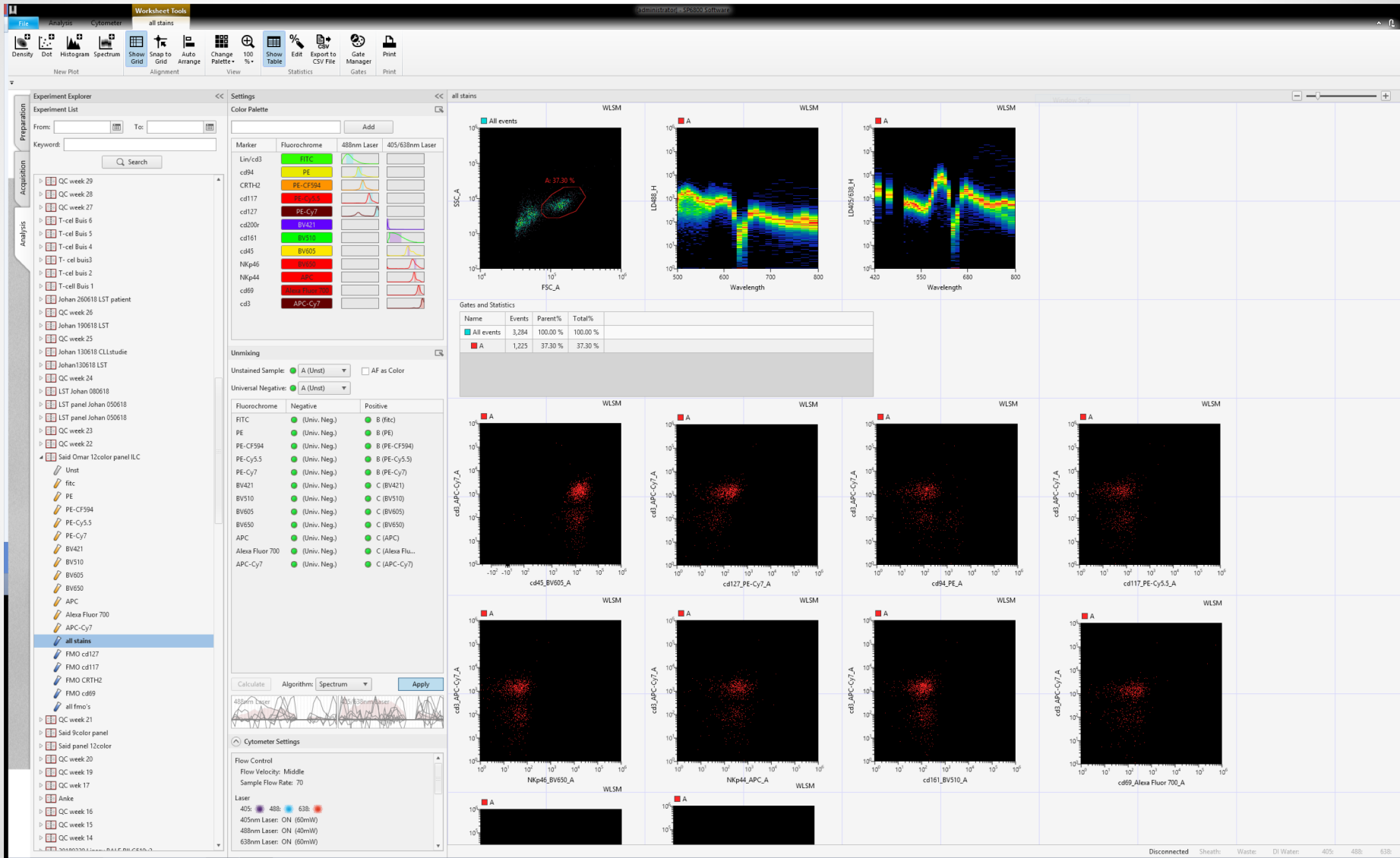
- Top Row: All events (SSC-A vs FSC-A), A (LD488_H vs Wavelength), A (LD488_H vs Wavelength)
- Middle Row: A (cd8_APC-Cy7_A vs cd45_BV605_A), A (cd8_APC-Cy7_A vs cd127_PE-Cy7_A), A (cd8_APC-Cy7_A vs cd84_PE_A), A (cd8_APC-Cy7_A vs cd117_PE-Cy5.5_A)
- Bottom Row: A (cd8_APC-Cy7_A vs NKp46_BV650_A), A (cd8_APC-Cy7_A vs NKp44_APC_A), A (cd8_APC-Cy7_A vs cd161_BV510_A), A (cd8_APC-Cy7_A vs cd69_Alexa Fluor 700_A)

Monitor
Event Rate: 0 KEPS
Flow Point: 0

Detector & Threshold
Window Extension: Normal
Threshold CH: FSC Threshold Value (%): 16.7
FSC Gain: 17 SSC Gain: 17
Fluorescence PMT Voltage (%): 62.0

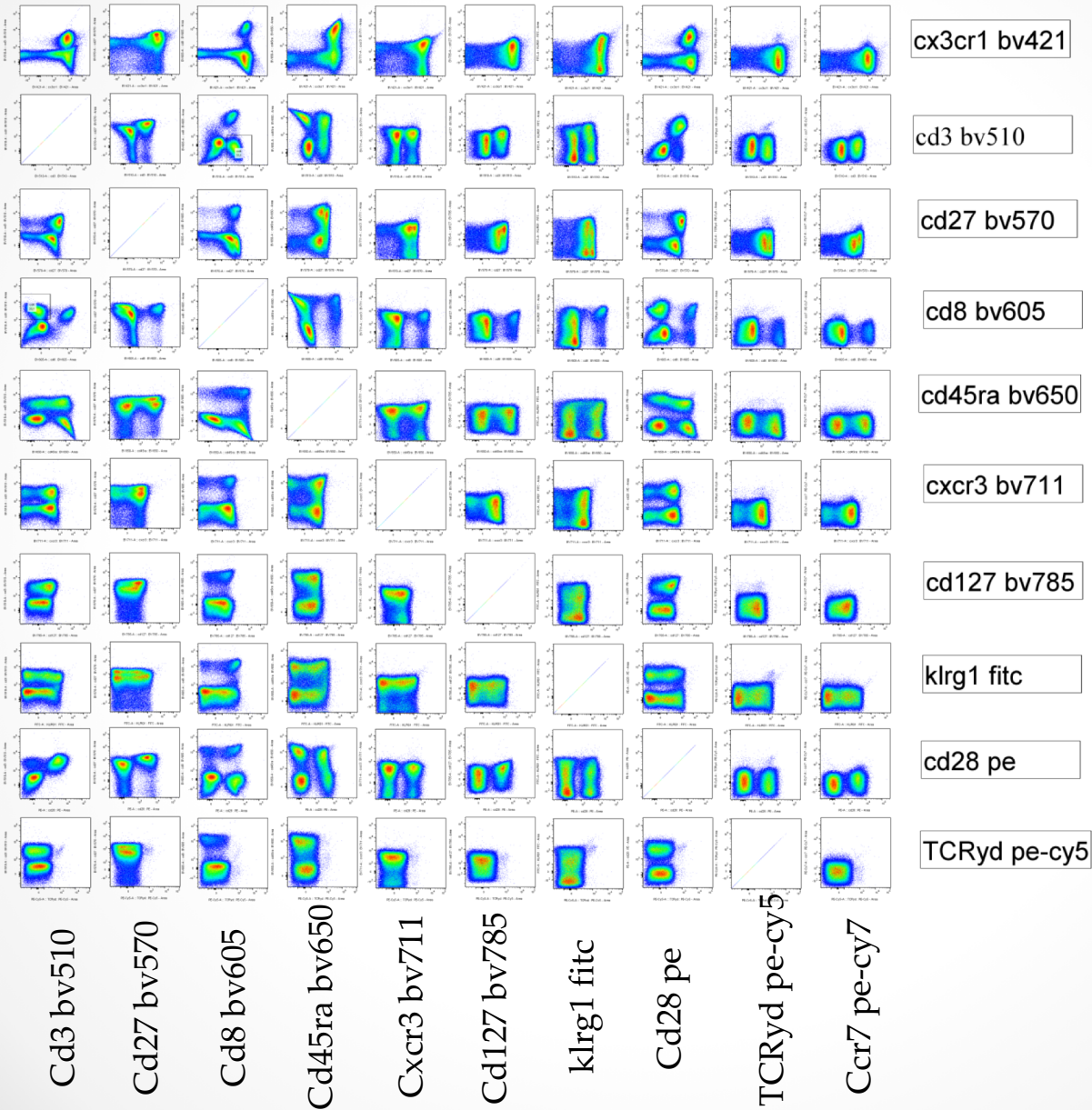
Status Bar: Ready Sheath: Waste DI Water: 40% 488: 638:

Sony SP6800 Software: analysis mode

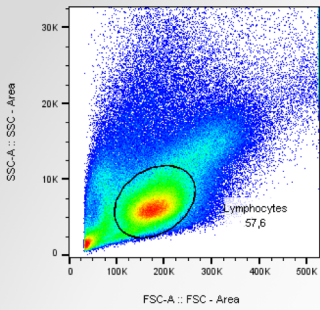


11 color experiment Violet & blue laser excited

tube 22

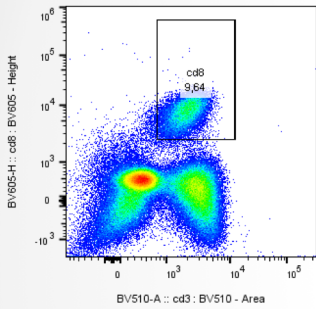


11 color experiment Violet & blue laser excited

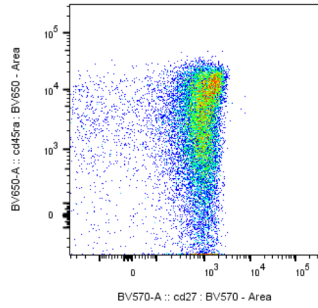


007 Tube - 21 WLSM.fcs
Ungsted
300000

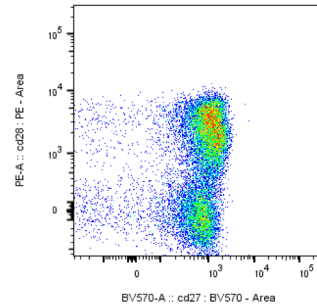
cd8+



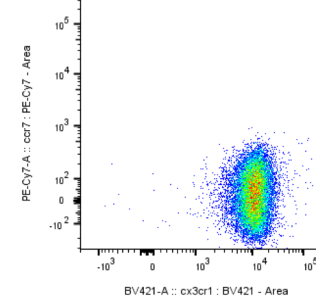
008 Tube - 22 WLSM.fcs
Lymphocytes
174627



008 Tube - 22 WLSM.fcs
cd8
16831



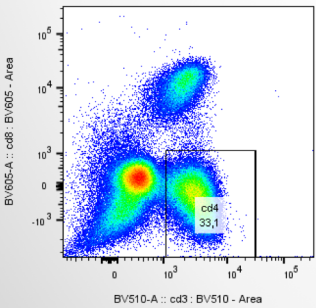
008 Tube - 22 WLSM.fcs
cd8
16831



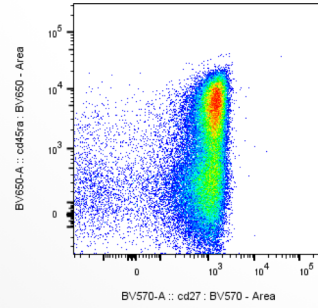
008 Tube - 22 WLSM.fcs
cd8
16831

| | |
|--------|---------|
| cx3cr1 | bv421 |
| cd3 | bv510 |
| cd27 | bv570 |
| cd8 | ef605nc |
| cd45ra | bv650 |
| cxcr3 | bv711 |
| cd127 | bv785 |
| klrg1 | fitc |
| cd28 | pe |
| TCRyd | pe-cy5 |
| ccr7 | pe-cy7 |

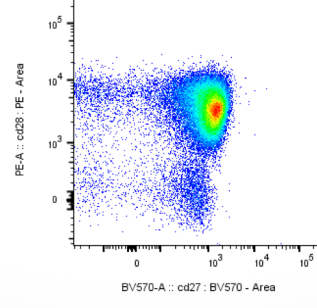
cd4+



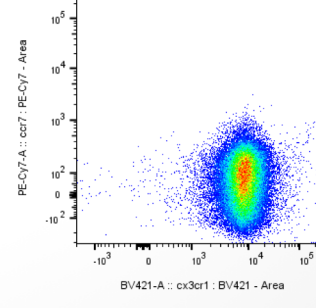
008 Tube - 22 WLSM.fcs
Lymphocytes
174627



008 Tube - 22 WLSM.fcs
cd4
57788

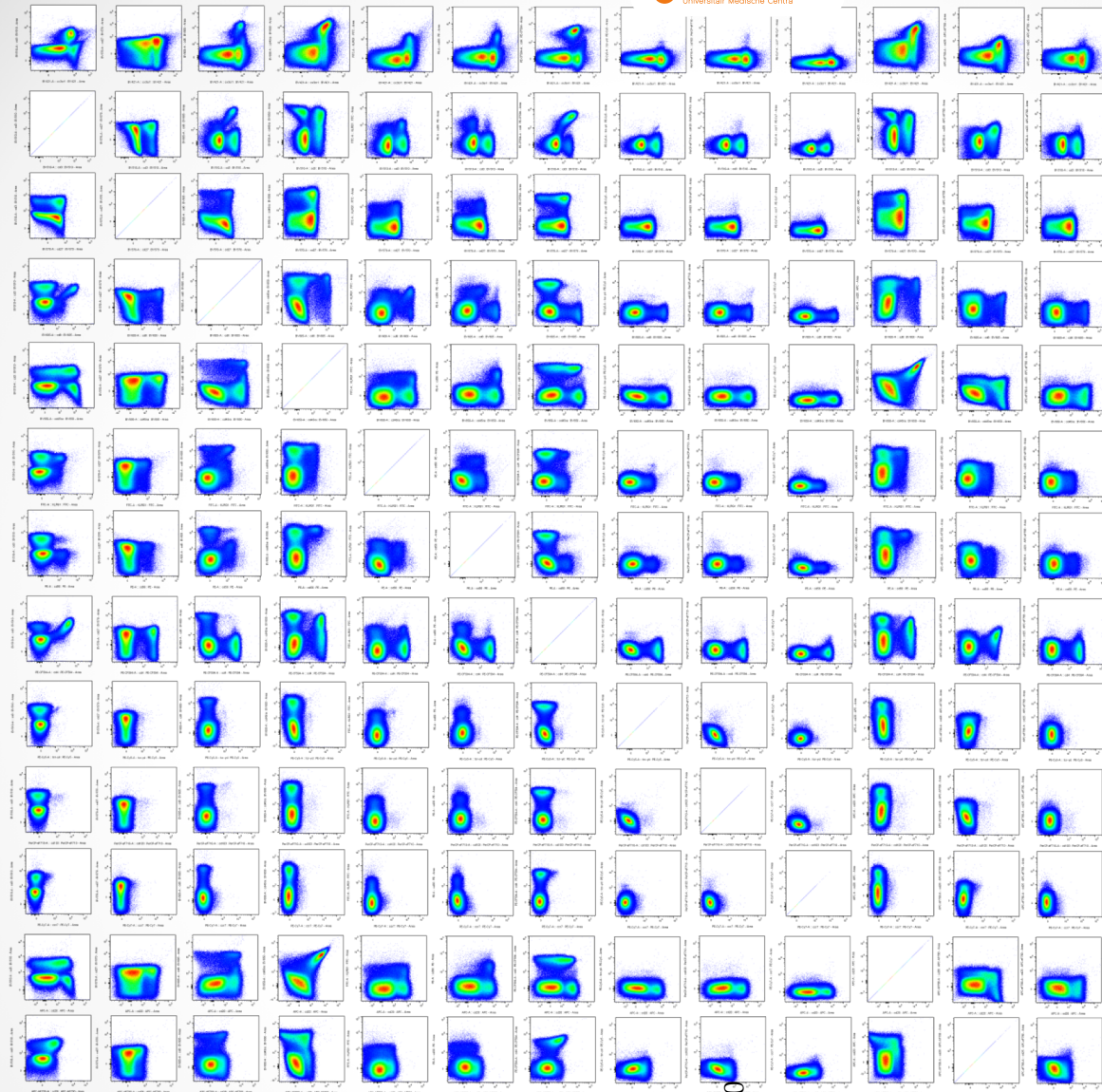


008 Tube - 22 WLSM.fcs
cd4
57788



008 Tube - 22 WLSM.fcs
cd4
57788

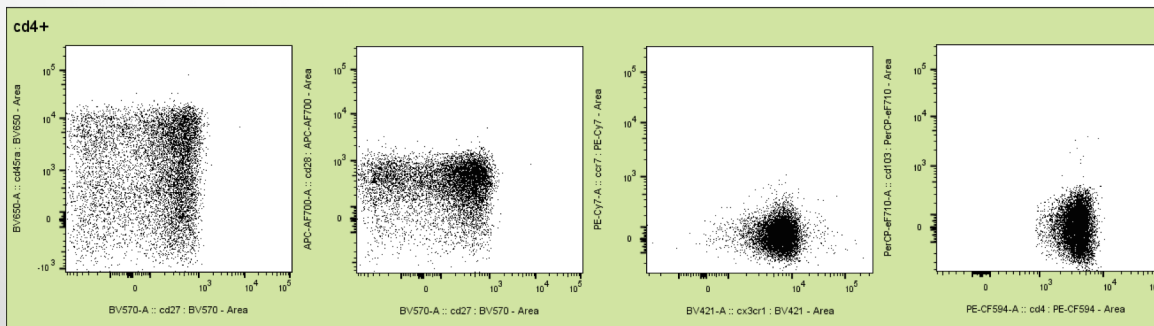
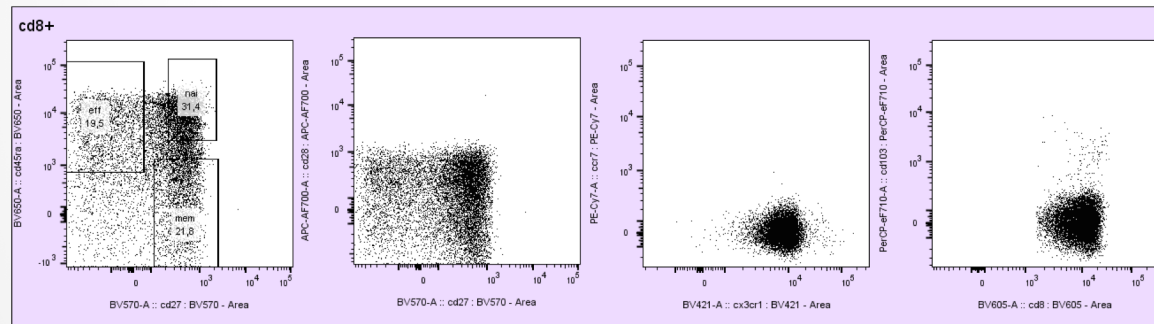
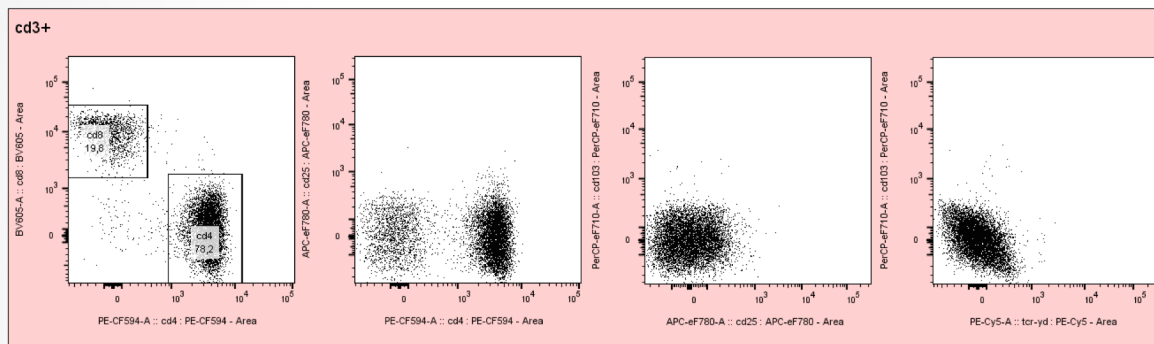
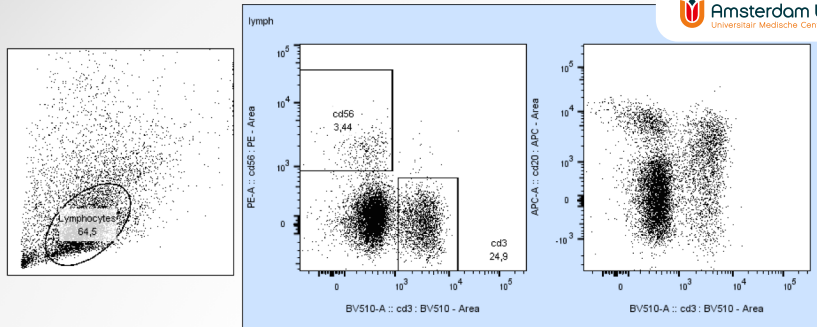
14 colors violet/blue/red excited



- Cx3cr1 bv421
- Cd3 bv510
- Cd27 bv570
- Cd8 bv605
- Cd45ra bv650
- klrg1 fitc
- Cd56 pe
- Cd4 pe-cf594
- Tcr-yd pe-cy5
- Cd103 percp-ef710
- Ccr7 pe-cy7
- Cd20 apc
- Cd28 apc-af700

- Cd3 bv510
- Cd27 bv570
- Cd8 bv605
- Cd45ra bv650
- klrg1 fitc
- Cd56 pe
- Cd4 pe-cf594
- Tcr-yd pe-cy5
- Cd103 percp-ef710
- Ccr7 pe-cy7
- Cd20 apc
- Cd28 apc-af700
- Cd25 apc-ef780

14 colors violet/blue/red excited



| | |
|--------|-------------|
| cx3cr1 | bv421 |
| cd3 | bv510 |
| cd27 | bv570 |
| cd8 | bv605 |
| cd45ra | bv650 |
| klrg1 | fitc |
| cd56 | pe |
| cd4 | pe-cf594 |
| TCRyd | pe-cy5 |
| cd103 | percp-ef710 |
| ccr7 | pe-cy7 |
| cd20 | apc |
| cd28 | apc-af700 |
| cd25 | apc-ef780 |

Eigenschappen Spectraal Analyse:

- Spectraal analyse geeft veel informatie over hoe fluorochromen zich gedragen (ook handig naast conventionele FCM). Denk aan excitatie door meerdere lasers en de kwaliteit van tandemconjugaten
- corrigeren voor autofluorescentie op basis van het unieke AF-spectrum
- Problemen met unmixing kunnen ontstaan wanneer fluorochromen dubbel excitatie vertonen (bv Qdots) en/of in het rood emitteren op violet & rode laser (door de collineair violet& rode lasers)
- Jammer genoeg heeft de Sony SP6800 geen UV laser voor het gebruik van UV-geexciteerde fluorochromen
- samenstellen van een kleuren panel zonder dat de piek emissies “passen” bij de optische configuratie van het instrument, dus geen filters of detector configuratie aanpassen. Instrument heeft 1 configuratie voor golflengtebereik $\pm 400-800\text{nm}$.
- Flexibiliteit in panel design. Bestaand panel eenvoudig uit te breiden met extra kleur(en) zonder dat dit invloed heeft op de andere kleuren in het panel
- het ontbreken van een compensatie algoritme. Hiervoor in de plaats komt een algoritme dat in de mix van fluorescente kleuren “zoekt” naar de unieke fingerprint van het gebruikte fluorochroom
- Instrument is zeer eenvoudig te gebruiken, software volgt in grote lijnen de gebruikelijke interface technieken (microsoft Office ribbon)

Sony Spectral Analyzer voor Diagnostiek van Lymfoproliferatieve Ziekten

Euroflow panel met 3 samples: *Normaal, B-cel monoklonaliteit, CLL*

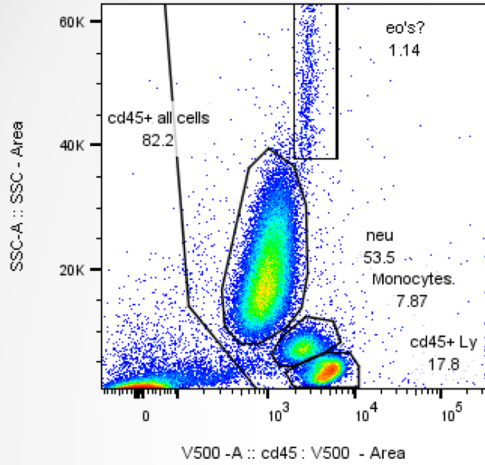
LST Lymphoid Screening Tube

| FITC | | PE | | PerCP-Cy5.5 | PE-Cy7 | APC | APC-H7 | V450/ PB | V500 | | | | | | |
|------------|------|-----------|------|-------------|--------|-------|--------|----------|--------|------|------|------|--------|------|------|
| smlgLambda | 5 µl | smlgKappa | 5 µl | CD5 | 15 µl | CD19 | 5 µl | CD3 | 2,5 µl | CD38 | 3 µl | CD4 | 0,5 µl | CD45 | 5 µl |
| CD8 | 5 µl | CD56 | 5 µl | | | TCRγδ | 3 µl | | | | | CD20 | 1 µl | | |

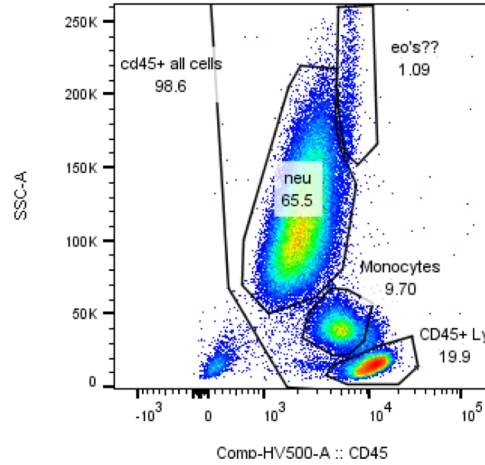
Hetzelfde sample is gemeten op de Sony SP6800 en de FacscantoII

Sample: norm

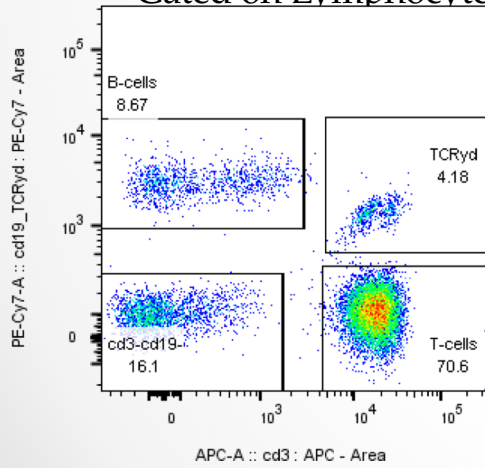
Sony SP6800



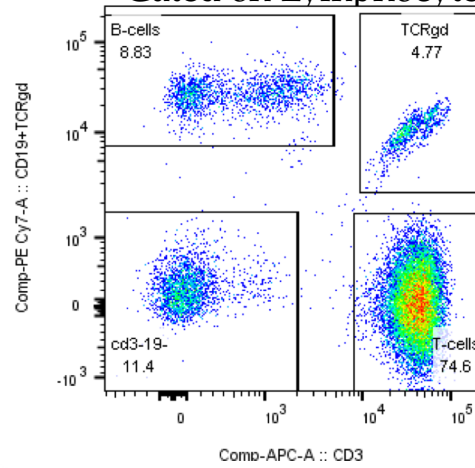
FacsantoII



Gated on Lymphocytes



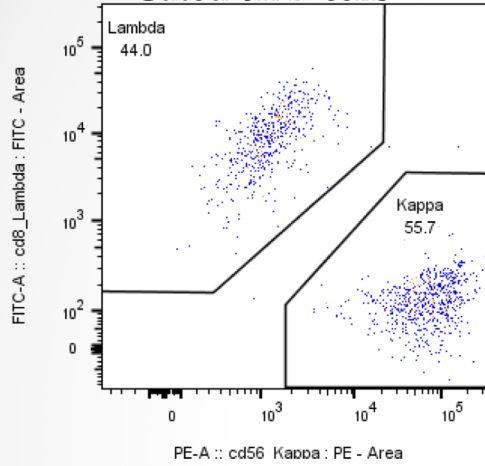
Gated on Lymphocytes



Sample: norm

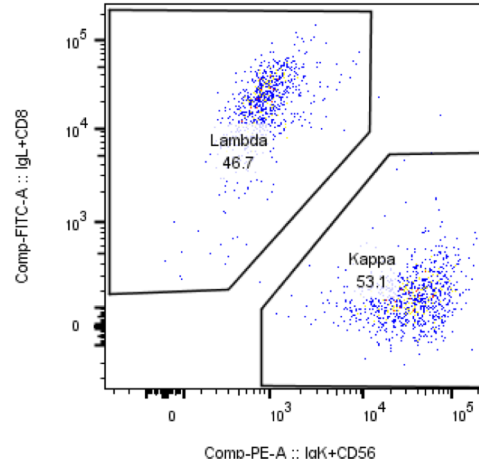
Sony SP6800

Gated on B-cells

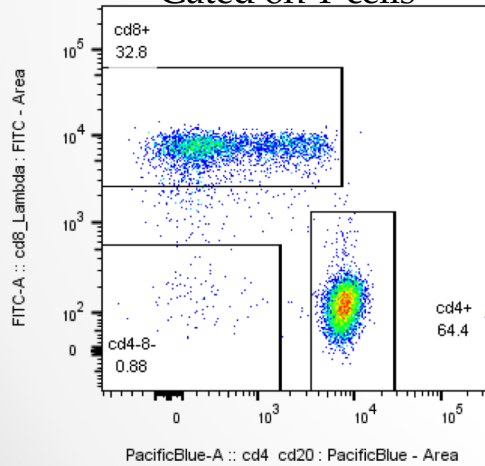


FacsantoII

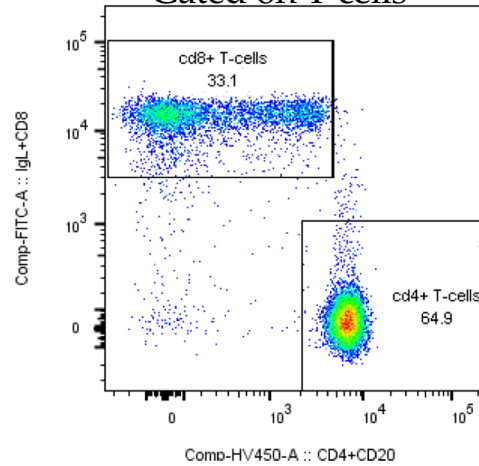
Gated on B-cells



Gated on T-cells

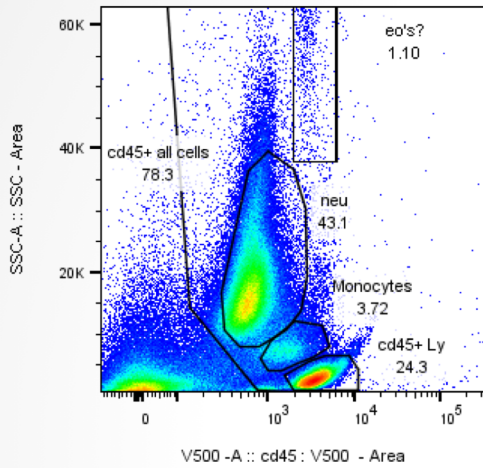


Gated on T-cells

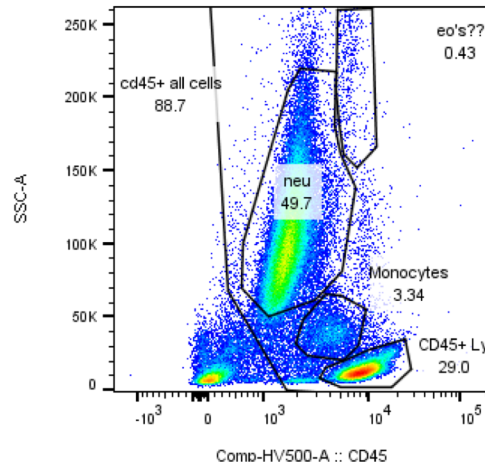


Sample: B cel monoclonaliteit

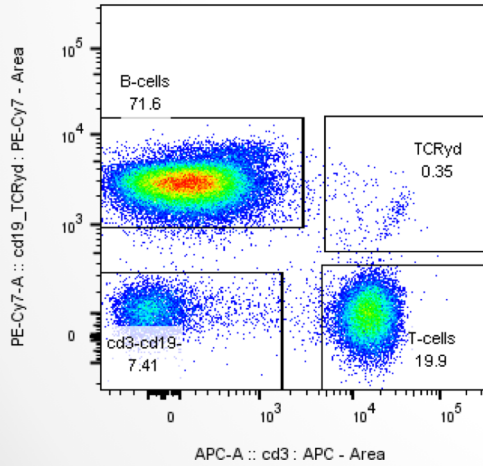
Sony SP6800



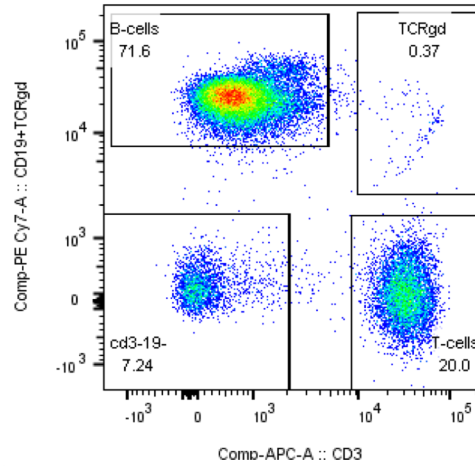
FacsantoII



Gated on Lymphocytes



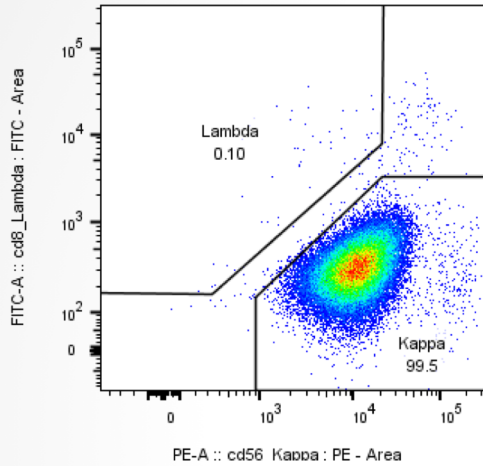
Gated on Lymphocytes



Sample: B cel monoclonaliteit

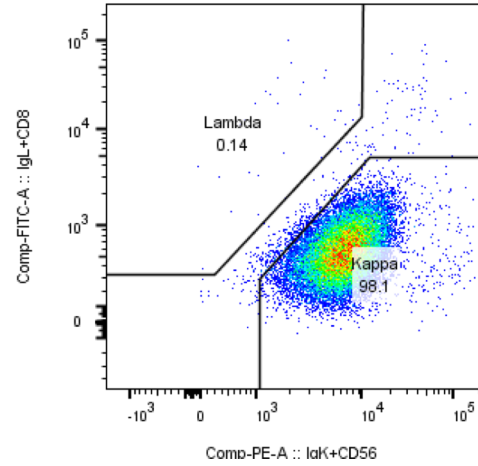
Sony SP6800

Gated on B-cells

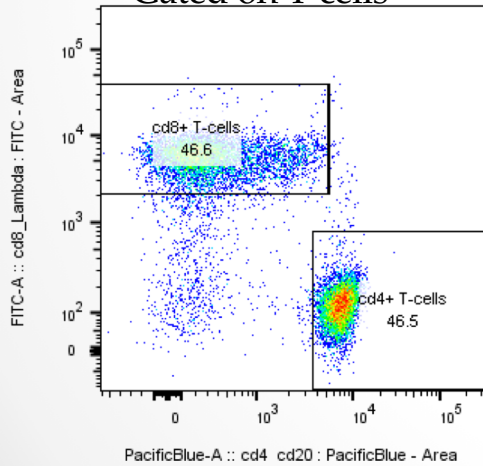


FacsantoII

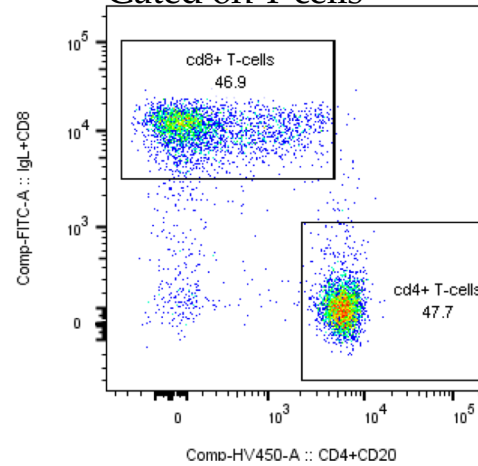
Gated on B-cells



Gated on T-cells

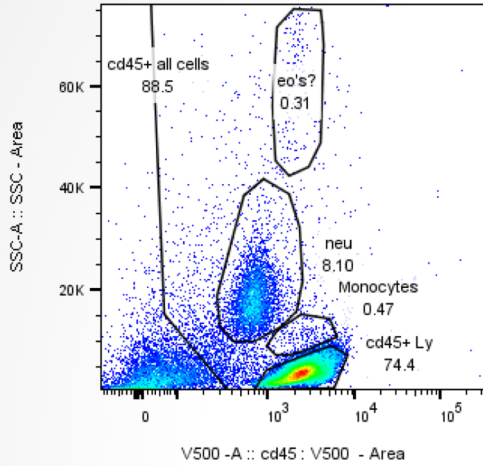


Gated on T-cells

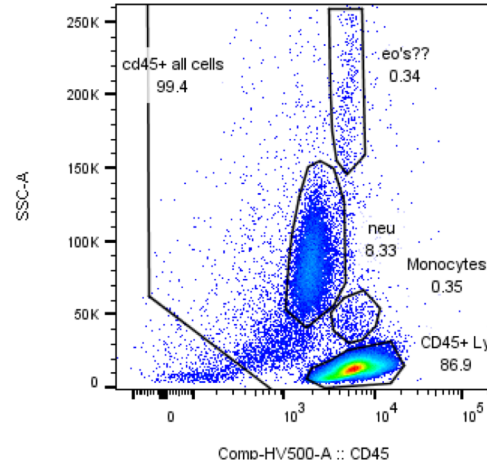


Sample: CLL

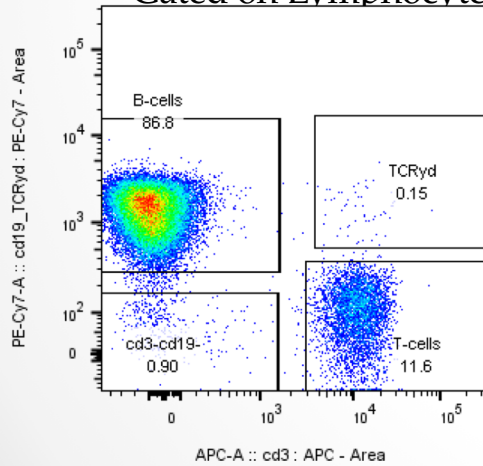
Sony SP6800



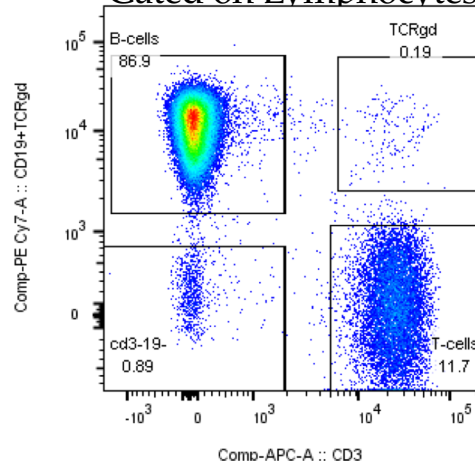
FacscantoII



Gated on Lymphocytes



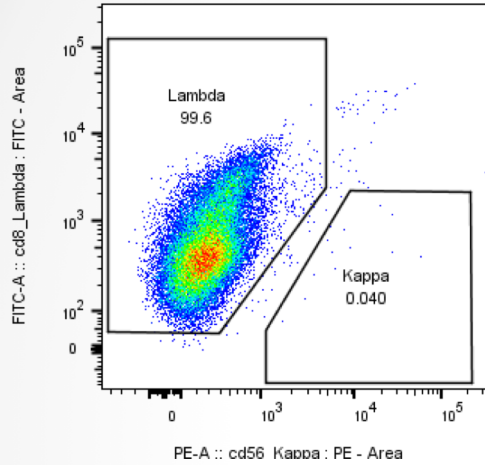
Gated on Lymphocytes



Sample: CLL

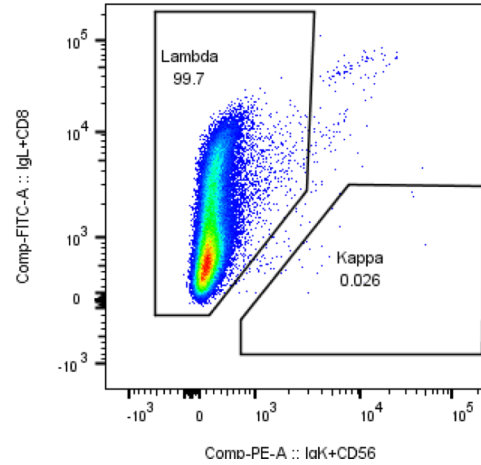
Sony SP6800

Gated on B-cells

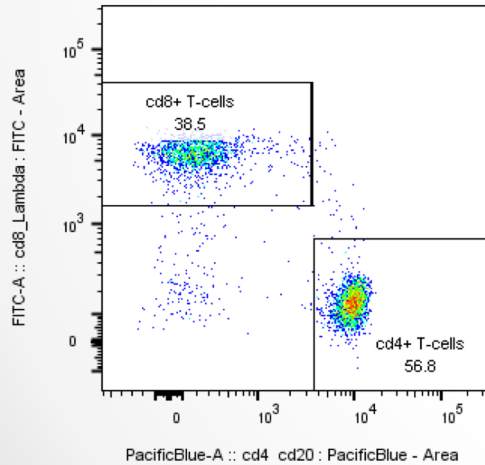


FacsantoII

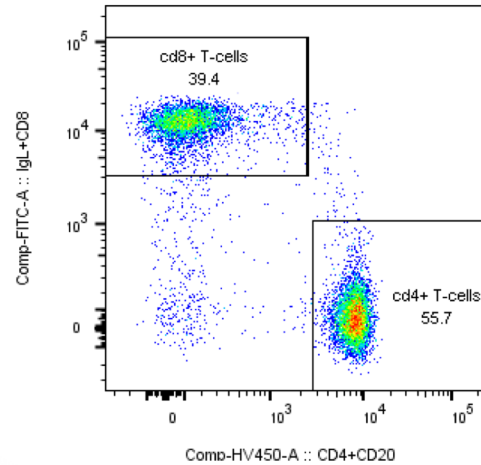
Gated on B-cells



Gated on T-cells



Gated on T-cells



Conclusies mbt de analyse van deze 3 samples en het Euroflow panel op de SP6800:

- Dotplots vertonen over het algemeen dezelfde populaties
- Sony SP6800 heeft andere dynamiek in scatter plot
- Populatie frequenties komen goed overeen
- Euroflow LST Panel is prima te analyseren met de SP6800
- Sony SP6800 lijkt iets minder gevoelig op pe-cy7

Ontwikkelingen in de Spectraal Flow Cytometrie

Naast de Sony spectral analyzers SP6800 & SP3800 is de firma Cytek Biosciences actief met oa de Aurora & NL3000

Kenmerken Cytek Aurora & NL3000:

- 3-5 lasers (gescheiden laserspots)
- Spectrum, 16 kanalen per laser
- APD solid state detectors



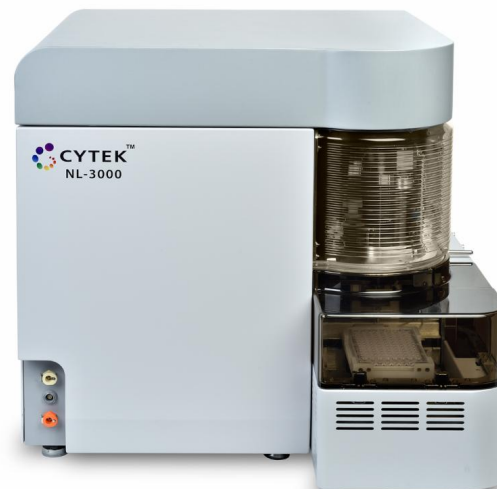
SP6800



SP3800



Aurora



NL3000

Dank aan:

Johan Dobber, Lab Speciele Hematologie, AMC

Mark Dessing, Sony Biotechnology