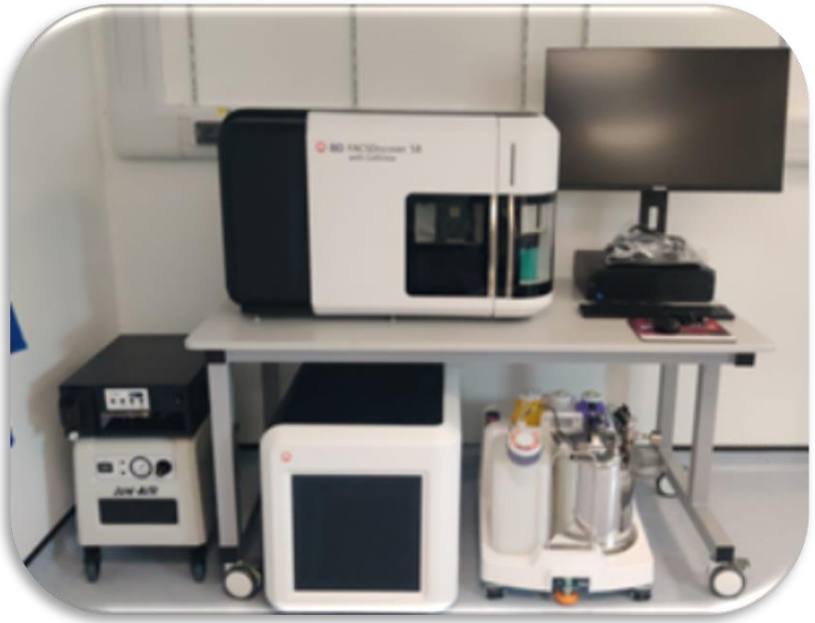
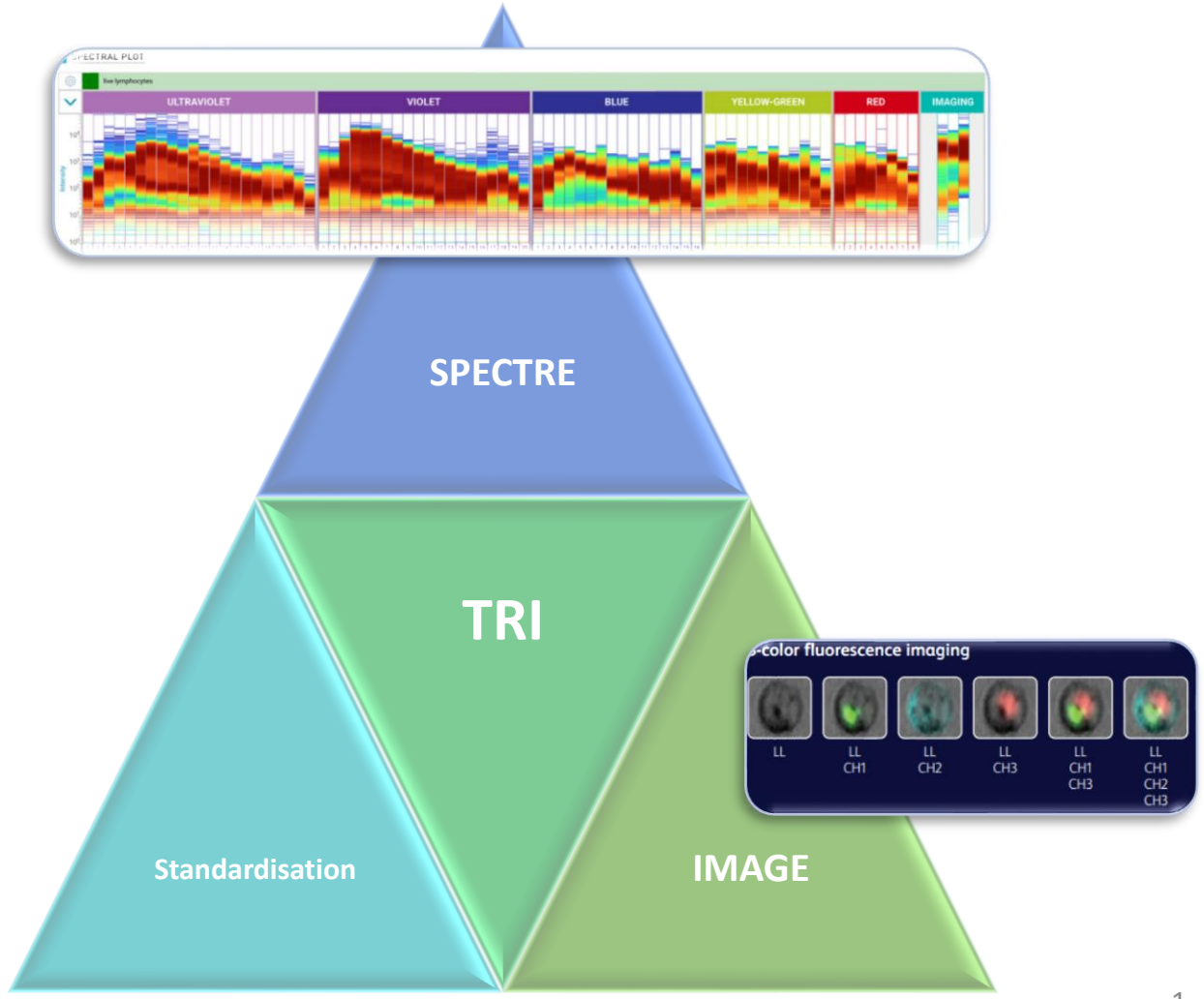
BD FACSDiscover ™ S8





Julie Cazareth, IPMC, CNRS, Valbonne

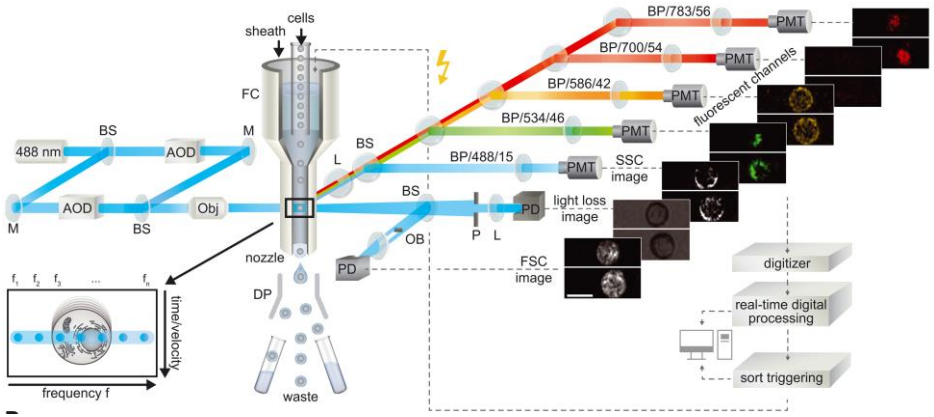
Pierre Grenot, U1109, INSERM, Strasbourg

AFC, Groupe FSC , 26 Janvier 2024

BD FACSDiscover™ S8 - OVERVIEW

OVERVIEW

- Base fluidique classique avec chariot simplifié
- Tout d'un trieur BD Biosciences !
- BD FACSChorus™ acquisition software
- Caisson optique/électronique déporté
- 86 détecteurs (PMT, APD et PD)
- Buses 85, 100 et 130µ (7psi)
- Gain calibré et optimisé de tous les détecteurs



Daniel Schraivogel et al. ,High-speed fluorescence image–enabled cell sorting.Science375,315-320(2022).DOI:10.1126/science.abj3013

BD FACSDiscover™ S8 - MODULE IMAGERIE

- Image recomposée

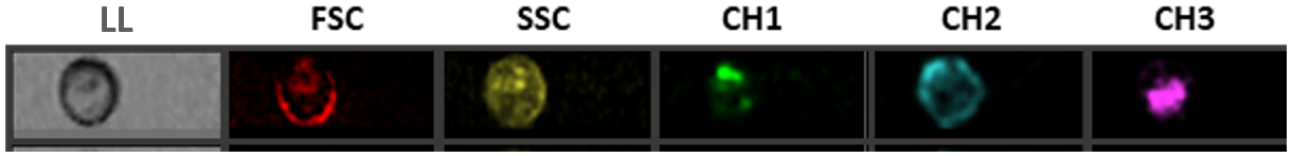
- 3 scatter (2 PDs and 1 PMT) sur le laser 488 nm
- 2 scatter (1 PDs and 1 PMT) sur le laser 405 nm

- 3 PMT couleurs sur 488
  - Image Channel 1 = 534/46
  - Image Channel 2 = 598/60
  - Image Channel 3 = 788/225

- Tri sur paramètres paramètres images

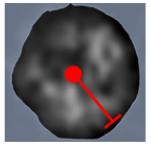
- Warning « panel design »

BD FACSDiscover™ S8 - MODULE IMAGERIE

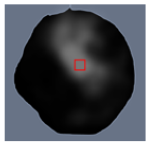


Size (LL,FSC, SSC, CH1, CH2, CH3)

Eccentricity (LL,FSC, SSC, CH1, CH2, CH3)



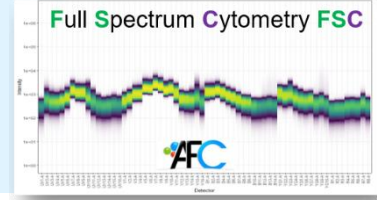
Radial moment (LL,FSC, SSC, CH1, CH2, CH3)



Max intensity (LL,FSC, SSC, CH1, CH2, CH3)

Correlation (CH1, CH2, CH3)
Delta center of mass (LL,FSC, SSC, CH1, CH2, CH3)
Diffusivity (LL,FSC, SSC, CH1, CH2, CH3)

BD FACSDiscover™ S8 - MODULE IMAGERIE



Save the date
22 mars à 13h30
Webinar Diana Ordonez
Co-organisation groupe cytométrie en image

RESEARCH TECHNOLOGY

High-speed fluorescence image–enabled cell sorting

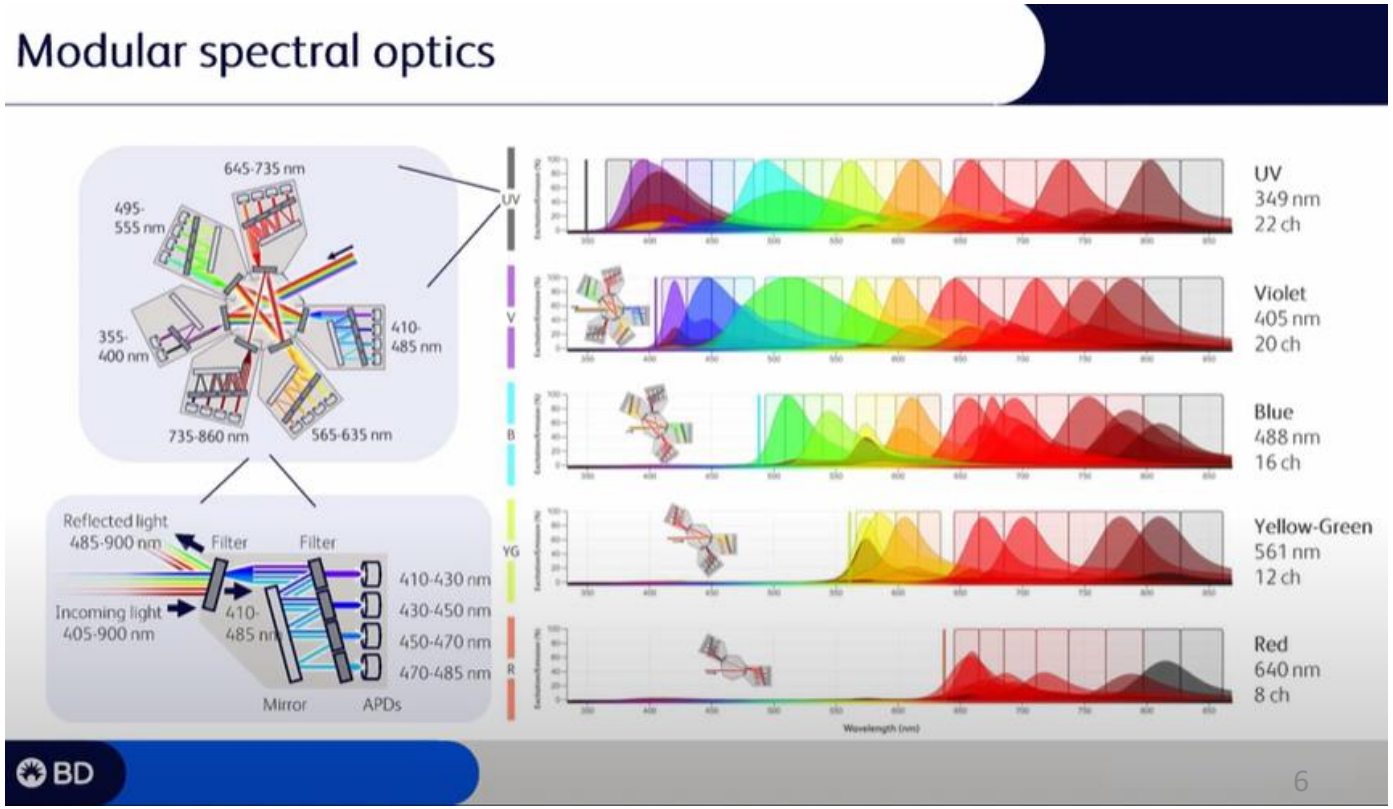
Daniel Schraivogel[1], Terra M. Kuhn[2]†, Benedikt Rauscher[1]†, Marta Rodríguez-Martínez[1]†, Malte Paulsen[3]‡, Keegan Owsley[4], Aaron Middlebrook[4], Christian Tischer[5], Beáta Ramasz[3], Diana Ordoñez-Rueda[3], Martina Dees[2], Sara Cuylen-Haering[2]*, Eric Diebold[4]*, Lars M. Steinmetz[1,6,7]*

Fast and selective isolation of single cells with unique spatial and morphological traits remains a technical challenge. Here, we address this by establishing high-speed image-enabled cell sorting (ICS), which records multicolor fluorescence images and sorts cells based on measurements from image data at speeds up to 15,000 events per second. We show that ICS quantifies cell morphology and localization of labeled proteins and increases the resolution of cell cycle analyses by separating mitotic stages. We combine ICS with CRISPR-pooled screens to identify regulators of the nuclear factor κB (NF-κB) pathway, enabling the completion of genome-wide image-based screens in about 9 hours of run time. By assessing complex cellular phenotypes, ICS substantially expands the phenotypic space accessible to cell-sorting applications and pooled genetic screening.

https://www.science.org/doi/epdf/10.1126/science.abj3013

« Full-spectrum fluorescence detection (365-860nm) »

- 78 APD sur 5 lasers avec bloc de 4 APD
- Bloc APD avec 1 fibre/laser
  - ➢ LP / mitroir /BP en séquentiel



Laser	Nbr APDs
Image	6
UV	22
Violet	20
Blue	16
YG	12
Red	8

BD FACSDiscover™ S8 Cell Sorter

Optical filter configuration

Laser	Detector name	Band pass filter
Ultraviolet	UV1 (375)	375/20
	UV2 (390)	392/14
	UV3 (420)	420/20
	UV4 (440)	440/20
	UV5 (460)	459/18
	UV6 (475)	476/18
	UV7 (500)	501/16
	UV8 (515)	517/16
	UV9 (530)	532/16
	UV10 (545)	547/16
	UV11 (575)	574/16
	UV12 (590)	590/16
	UV13 (605)	607/18
	UV14 (625)	625/18
	UV15 (655)	655/20
	UV16 (675)	675/20
	UV17 (700)	698/26
	UV18 (725)	724/26
	UV19 (750)	752/30
	UV20 (780)	782/30
	UV21 (810)	812/30
	UV22 (845)	844/24

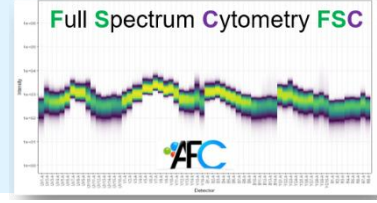
Laser	Detector name	Band pass filter
Violet	SSC (Violet)	405/15
	Lightloss (Violet)	405/5
	V1 (420)	420/20
	V2 (440)	440/20
	V3 (460)	459/18
	V4 (475)	476/16
	V5 (500)	501/16
	V6 (515)	517/16
	V7 (530)	532/16
	V8 (545)	547/16
	V9 (575)	574/16
	V10 (590)	590/16
	V11 (605)	607/18
	V12 (625)	625/18
	V13 (655)	655/20
	V14 (675)	675/20
	V15 (700)	698/26
	V16 (725)	724/26
	V17 (750)	752/30
	V18 (780)	782/30
	V19 (810)	812/30
	V20 (845)	844/34

Laser	Detector name	Band pass filter
Blue	B1 (500)	501/16
	B2 (515)	517/16
	B3 (530)	532/16
	B4 (545)	547/16
	B5 (575)	574/16
	B6 (590)	590/16
	B7 (605)	607/18
	B8 (625)	625/18
	B9 (655)	655/20
	B10 (675)	675/20
	B11 (700)	698/26
	B12 (725)	724/16
	B13 (750)	752/30
	B14 (780)	782/30
	B15 (810)	812/30
	B16 (845)	844/34

Laser	Detector name	Band pass filter
Imaging	FSC	N/A
	SSC (Imaging)	488/15
	Light Loss (Imaging)	488/5
	ImgB1 (535)	534/46
	ImgB2 (600)	600/60
	ImgB3 (790)	788/225

Laser	Detector name	Band pass filter
Yellow Green	YG1 (575)	574/16
	YG2 (590)	590/16
	YG3 (605)	607/18
	YG4 (625)	625/18
	YG5 (655)	655/20
	YG6 (675)	675/20
	YG7 (700)	698/26
	YG8 (725)	724/26
	YG9 (750)	752/30
	YG10 (780)	782/30
	YG11 (810)	812/30
	YG12 (845)	844/34

Laser	Detector name	Band pass filter
Red	R1 (655)	655/20
	R2 (675)	675/20
	R3 (700)	698/26
	R4 (725)	724/26
	R5 (750)	752/30
	R6 (780)	782/30
	R7 (810)	812/30
	R8 (845)	844/34



Spectral FX technology

Spectral Unmixing propriétaire (SpectralFX™ System-Aware unmixing; **base WLSM** implémentée avec certains paramètres, dont le résultat du QC)	La **réduction du spread** (par rapport à un unmixing classique OLS ou WLS) est liée quant à elle à une notion de variance analysée sur le single-stain unmixé.	**Maintien du unmixing** en temps réel si ajustement des gains

➢ System aware unmixing = « aware » du bruit de fond

DAILY QC = Combinaison du système de LED pulsé Quantiflash et de billes

1. Calibration des détecteurs indépendant du bruit optique par système

   Quantiflash = détermination des gains minimum (pas de billes à cette étape)
2. QC spot laser 488nm pour imagerie
3. Laser delay
4. Optimisation des gains
5. Accudrop

ORIGINAL ARTICLE

Cytometry PART A
Journal of the International Society for Advancement of Cytometry

MIFlowCyt

Determination of Background, Signal-to-Noise, and Dynamic Range of a Flow Cytometer: A Novel Practical Method for Instrument Characterization and Standardization

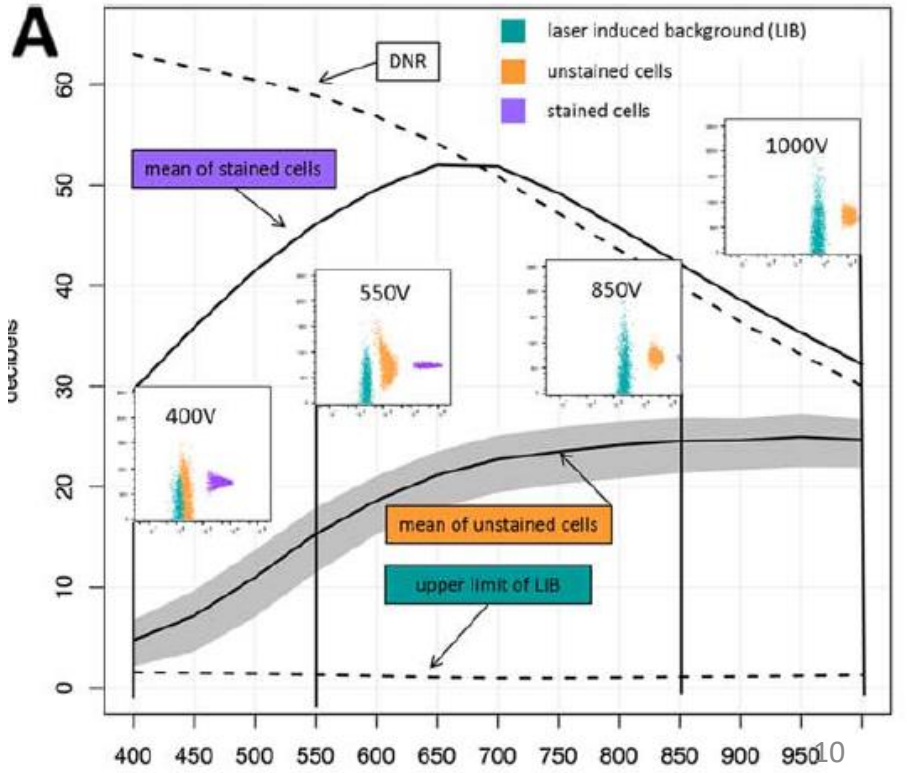
Claudia Giesecke,[1,2†] Kristen Feher,[3†] Konrad von Volkmann,[4‡] Jenny Kirsch,[3] Andreas Radbruch,[1] Toralf Kaiser[3*]

quantiFlash
Calibration Light Source for Cytometry and Low Light Detectors

LED Pulser (350-880nm)

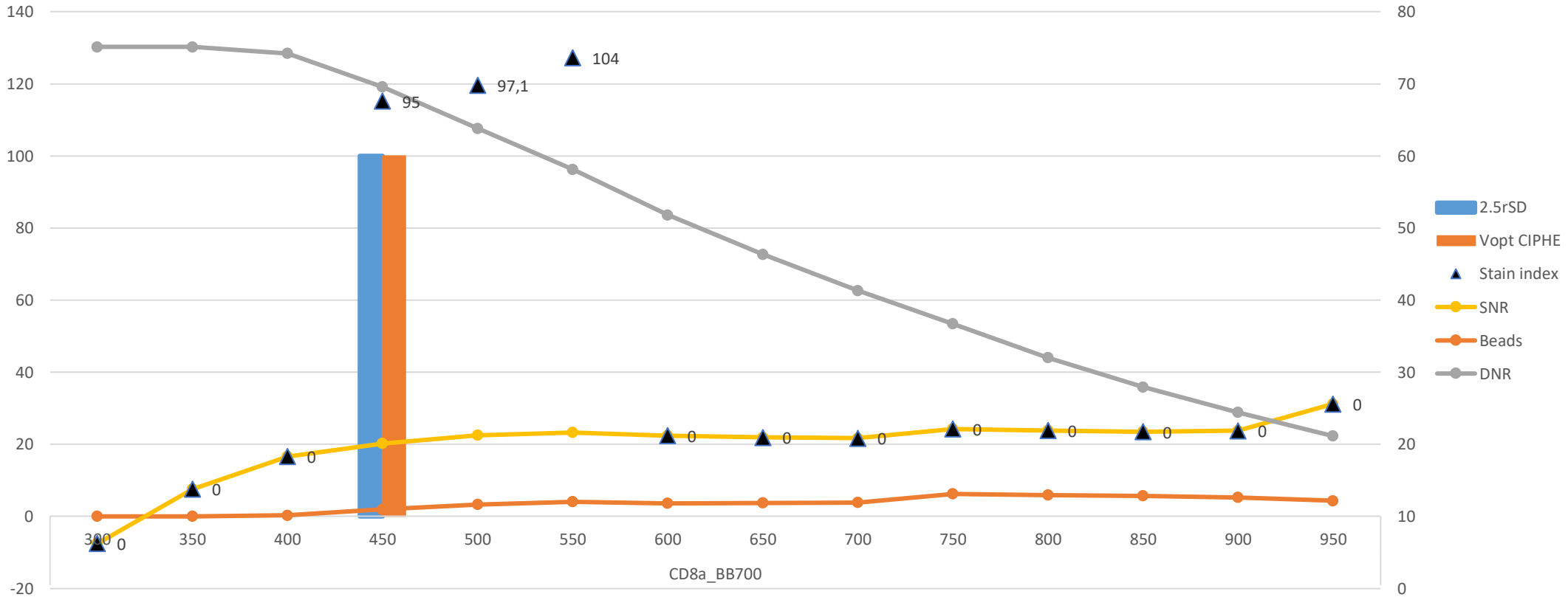
- Calibration sans billes
- Calcul
  - bruit de fond (noise)
  - Linéarité
  - Dynamic range

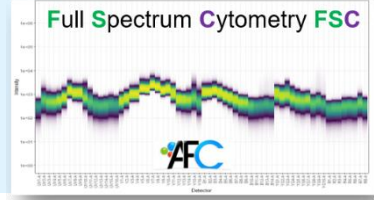
Impacte le calcul de la décomposition spectrale



Méthode développée au CIPHE sur LSRII Fortessa en 2021

LSR II (5 Lasers) 3 méthodes
- 2,5 rSD
- LED pulser
- Voltages optimaux



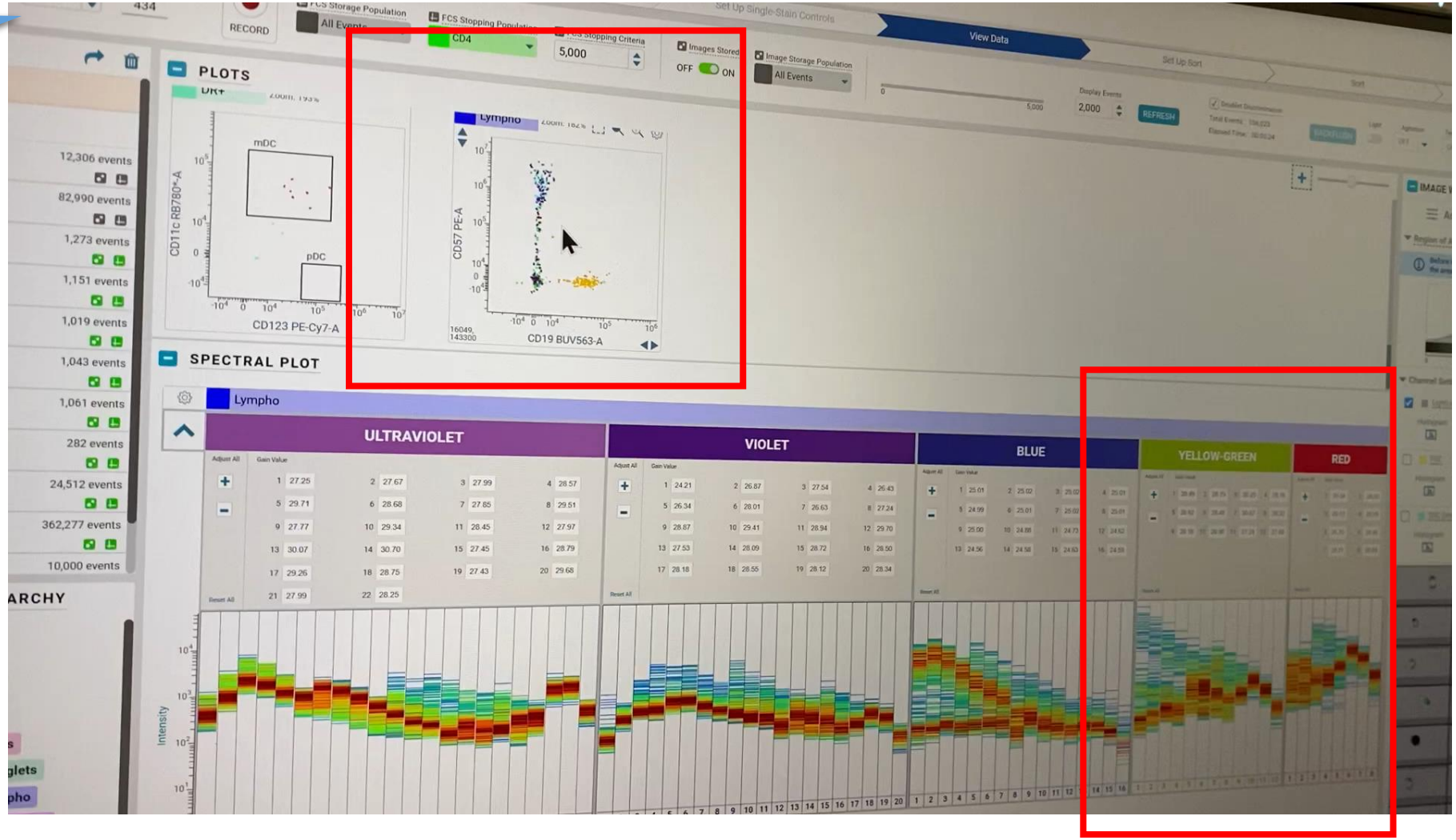


DAILY QC = Combinaison du système de LED pulsé Quantiflash et de billes

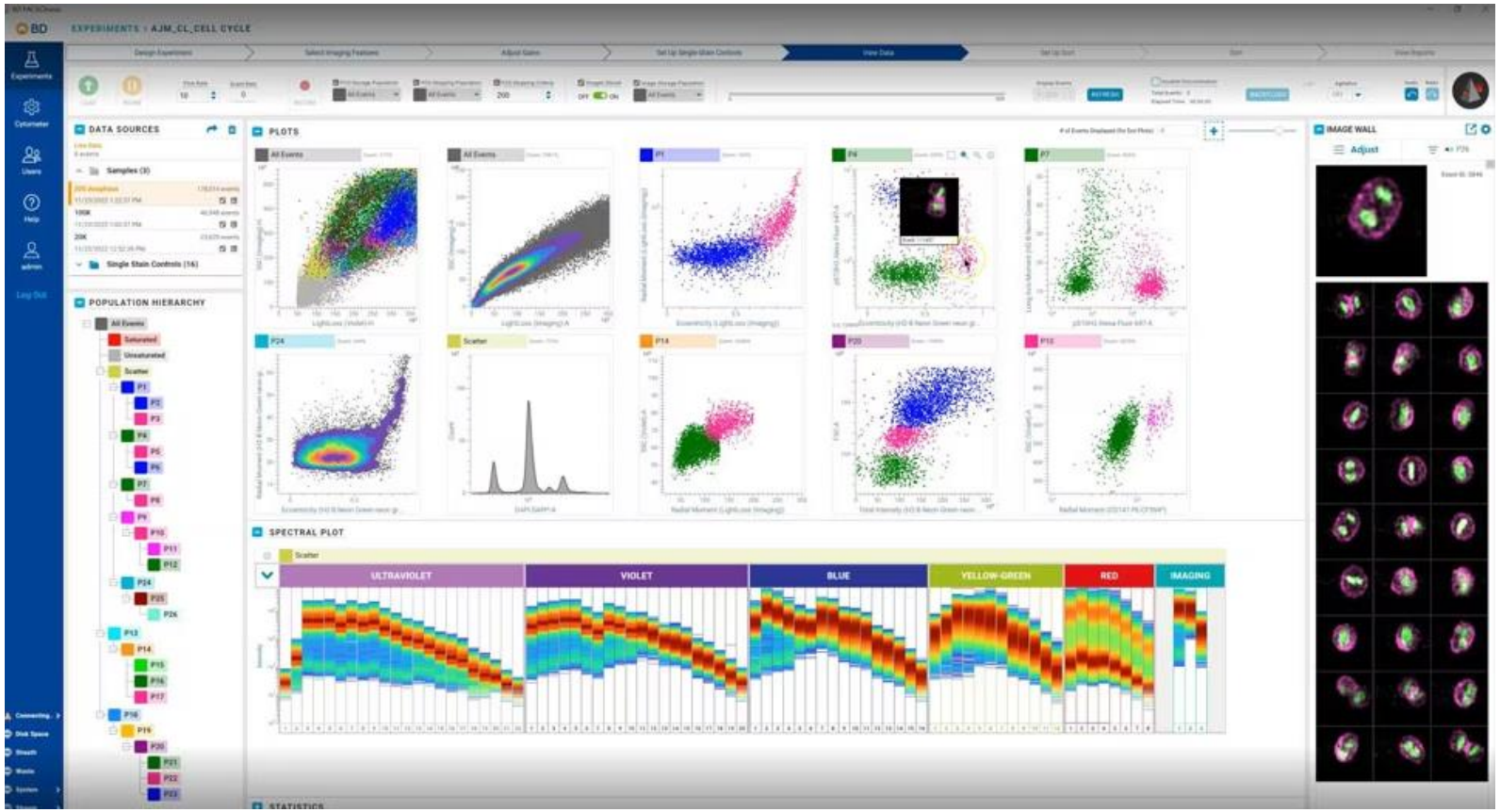
- Réduction du bruit de fond optique = réduction du spread
- Optimisation des gains des APDs
- Standardisation automatique
- Maintien du unmixing en « live » = « System aware unmixing »

BD FACSDiscover™ S8 - ACQUISITION ET QC

En live



« FACS Chorus acquisition software »



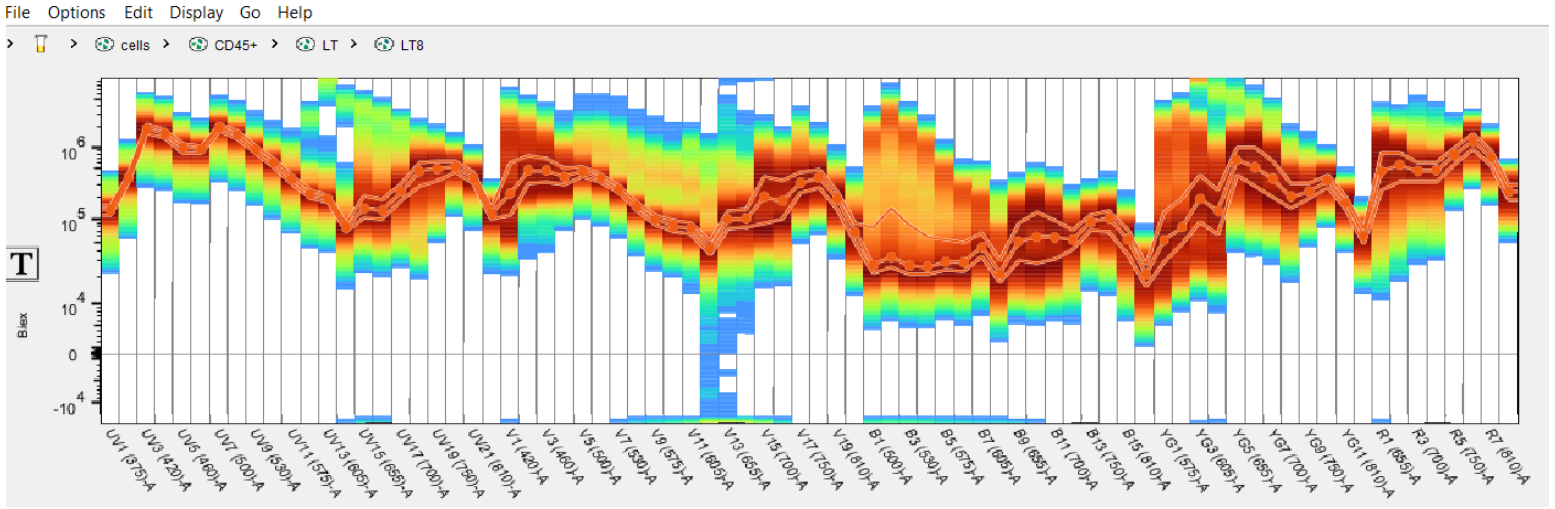
- Workflow acquisition simplifié
- AF extraction automatique (1seule)
- Unmixing immédiat
- Vérification sur matrice NxN dans FACSChorus

- Post-acquisition: re-comp dans FlowJo…

➢ Upgrade logiciel à venir

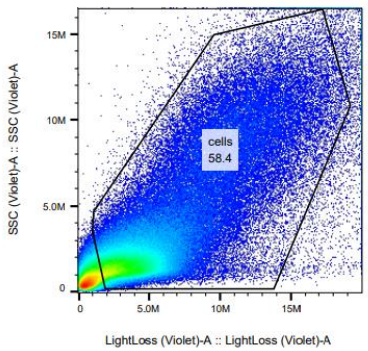
FLOWJO v10.10

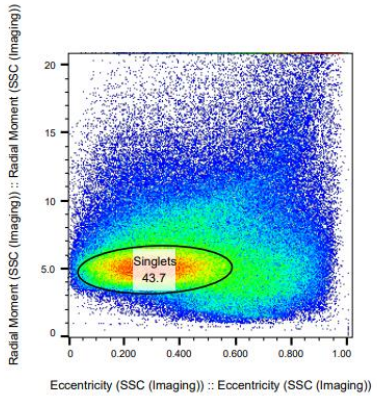
- Unmixing : SpectralFX™ unmixing ou “ordinary-least-squares (OLS)” traditionnel
- Compensation post-acquisition
- Visualisation des spectres et des images (plugin CellView Lens) !!

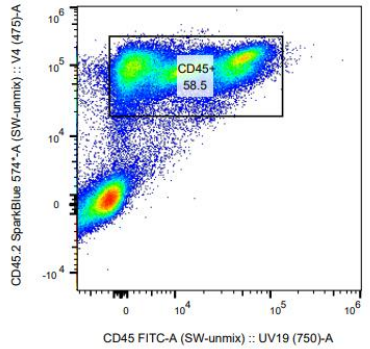


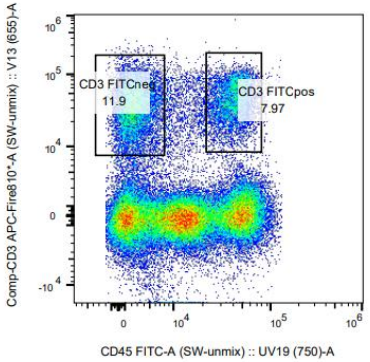
Données de Cyrille Mionnet (merci !!!)

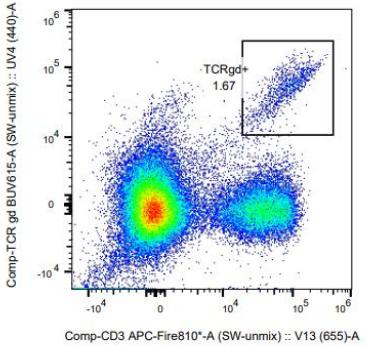
- Panel 36 couleurs, poumons de souris, extraction AF automatique
- Unmixing d'acquisition
- Compensation dans FlowJo

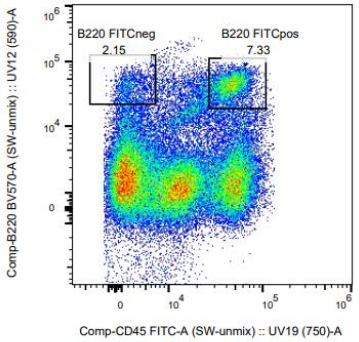


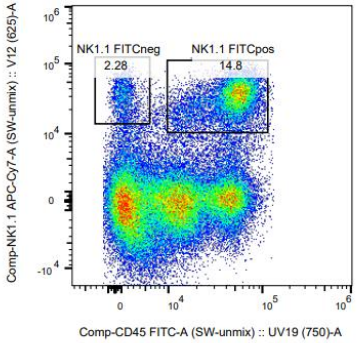


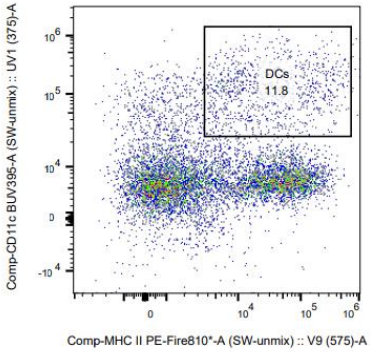


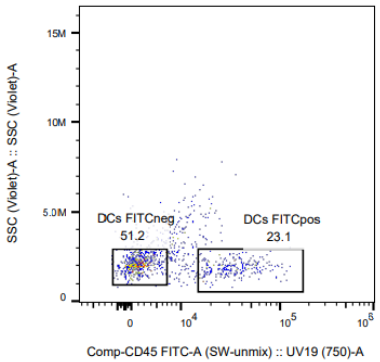




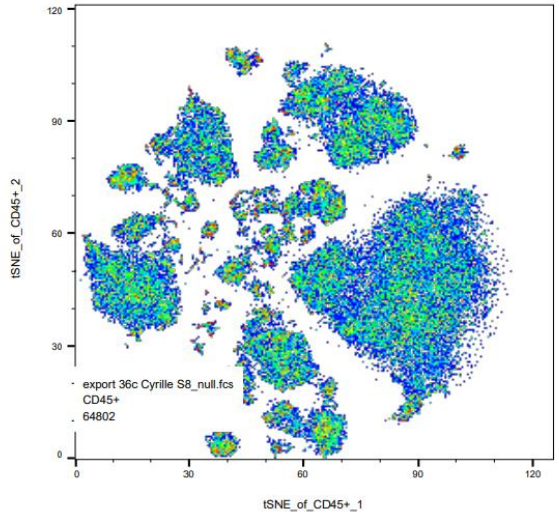


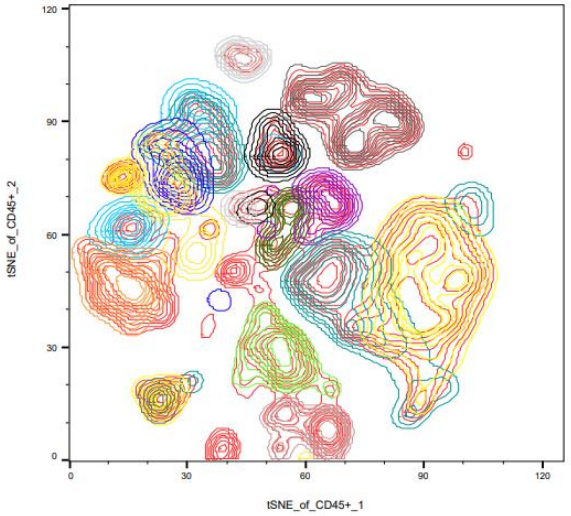


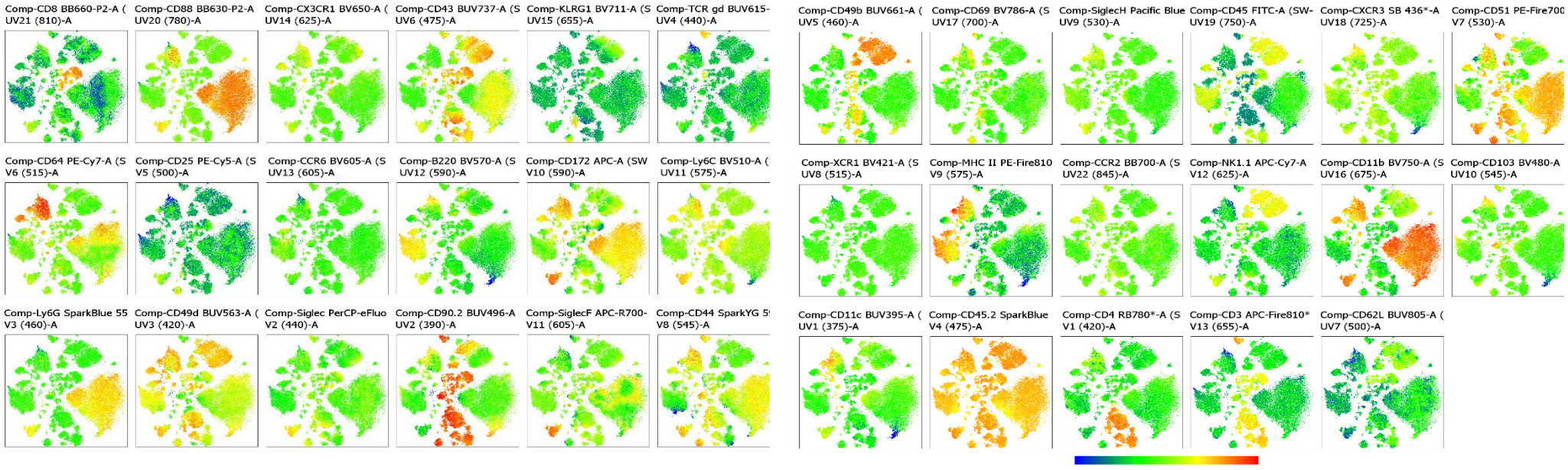




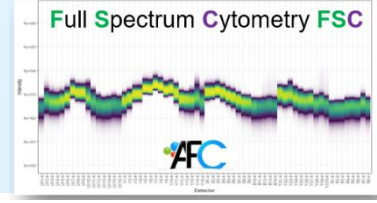
BD FACSDiscover™ S8 - DATAS







BD FACSDiscover™ S8 - DATAS



Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

New Results

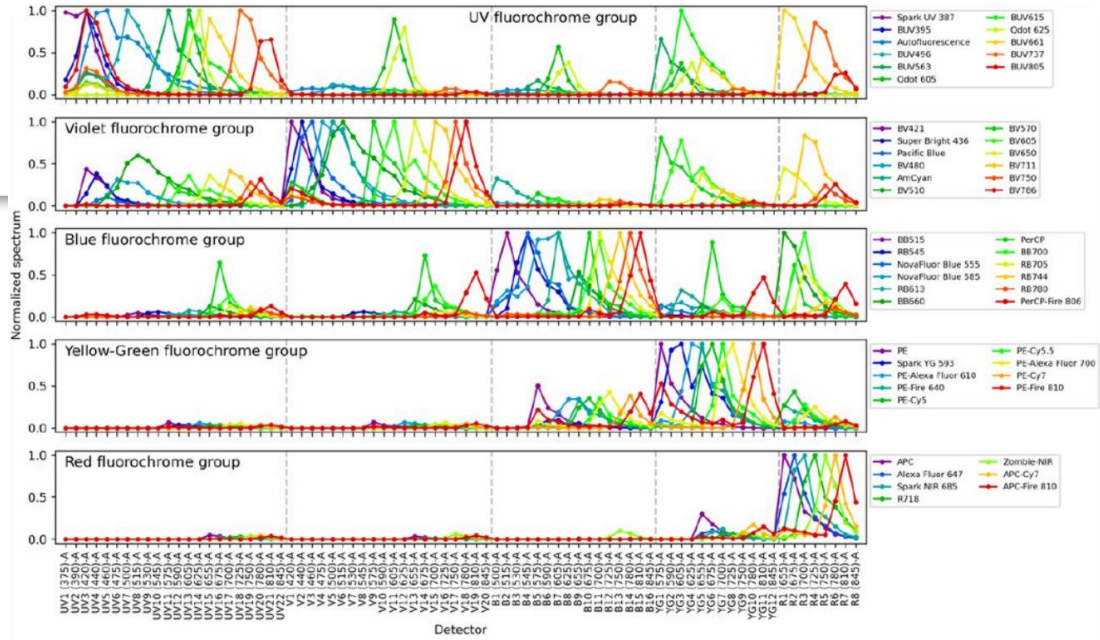
Follow this preprint

50-color phenotyping of the human immune system with in-depth assessment of T cells and dendritic cells

Andrew J. Konecny, Peter Mage, Aaron J. Tyznik, Martin Prlic, Florian Mair

doi: https://doi.org/10.1101/2023.12.14.571745

- ✓ **Hu PBMC**
- ✓ **Tri**
- ✓ **50 couleurs sur BD FACSDiscover S8**
- ✓ **45 couleurs sur Sony ID700**



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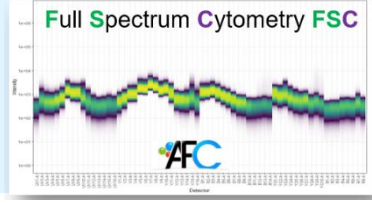

❖ **Webinar de Diana Ordonnez**, Head of Flow Cytometry, EMBL, Heidelberg

22 mars 2024 à 13h30

❖ Réunions du goupe FSC, les vendredis à 14h:

- **29 mars 2024**: Nathalie Jouy, Campus Santé de Lille
- **24 mai 2024**: Jean-François Mayol, Université de Lausanne

❖ **Journée thématique « High-content cytometry », le 13 juin 2024 à l'Institut Pasteur de Lille**

❖ Pensez à **renouveler vos adhésions pour 2024** !!! Vos « droits » de membres (et votre accès à la liste de diffusion des groupes) expireront fin Février….