

1



2

CLEAR IDEA about DATA GENERATION

- How much fluorescence
- Percentage of positive and negative
- How more positive is X than Y
- Ratio between 2 fluorescences
- Protein-protein interactions
- Cell-cell interaction
- Cytokines
- Cytoskeleton enzymes
- Transcription factors
- Signaling molecules
- (Phosphorylation)
- Monitor signaling cascades
- Proteins of apoptosis
- Activation of caspases
- Changes in the mitochondrial membrane potential
- Changes in the membrane
- Cell shrinkage
- Chromatin changes
- DNA degradation
- Ions fluxes (Ca^{++})
- Protein translocation
- Target-specific RNA
- MicroRNAs
- Gene expression
- High-dimensional data
- High-throughput screening

For every type of
DATA
there are instruments &
approaches more accurate
than others

3

KNOW your SAMPLES

Powerful tool to identify and analyse multiple parameters simultaneously on single particles

Any suspended particle/cell 0.2–150 μm sized is suitable for analysis

- Blood
- Hematopoietic cells
- Epithelial and stromal cells
- (Non)adherent cells from culture
- Waterborne microorganisms
- Bacteria
- Yeast
- Nanoparticles (EV)
- Tumor cells
- Micrometer-sized oil emulsion droplets (with enzymes)

For every type of
SAMPLES
there are proper instruments
and approaches

Make the right choice by
knowing samples properties

4

FIND THE CORRECT TOOLS

5

CHOOSING A PROPER INSTRUMENT

6

3

CONVENTIONAL FACS ANALYSERS

BD LSR Fortessa



Laser		Filter	
Blue 488nm	Bandpass Filter	Suggested Fluorochromes	
530/50		Brilliant Blue 515, FITC, Alexa Fluor 488, PerCP-Cy5.5,	
570/50			
Yellow/Green 565nm			
610/20	PE-Dazzle	PE-CF594	
710/50	PE-Cy5.5		
780/60	PE-Cy7		
Red 635nm			
670/20	APC, Alexa Fluor 647		
700/45		Anti-Rabbit IgG 700,	
780/60	APC-Alexa Fluor 700, DRAQ5, Zombe NIR		
Violet 405nm			
450/50	BV421, V450, DAPI, Pacific Blue		
525/50	BV510, Zombie Aqua, V500		
610/20	BV605		
700/20	BV700		
710/50	BV711		
780/60	BV786		
UV 355nm			
379/28	BUV95		
450/50	DAPI, Hoechst blue		
730/45	BUV373		

96well-plate loader

BD LSR II



Laser		Filter	
570/20	Brilliant Ultra Violet(BUV) 350, Indo-1 (Quench)	FITC	Alloxa Fluor® 488
525/50	DAPI, Hoechst, BUV421, Indo-1 (Quench)	PE-Horizon Red®	PerCP-Cy5.5
780/60	BUV711	Infrared	PE-Cy7
610/20			PerCP-Cy5.5
710/50			PE-Cy7
780/60			Alloxa Fluor® 647
450/50	BUV421, Indo-1 (Quench)	For Red	APC-Cy7
635/50		Infrared	BD APC-A7
405/50		Green	AntiCy™
560/20	BUV373, Indo-1 (Quench)	Blue	BD Horizon V600
780/60			PerCP-Cy5.5

BD FACS Canto II



Laser		Filter	
570/20	Brilliant Ultra Violet(BUV) 350, Indo-1 (Quench)	FITC	Alloxa Fluor® 488
525/50	DAPI, Hoechst, BUV421, Indo-1 (Quench)	PE-Horizon Red®	PerCP-Cy5.5
780/60	BUV711	Infrared	PE-Cy7
610/20			PerCP-Cy5.5
710/50			PE-Cy7
780/60			Alloxa Fluor® 647
450/50	BUV421, Indo-1 (Quench)	For Red	APC-Cy7
635/50		Infrared	BD APC-A7
405/50		Green	AntiCy™
560/20	BUV373, Indo-1 (Quench)	Blue	BD Horizon V600
780/60			PerCP-Cy5.5

BC CytoFLEX S



Laser		Filter	
450 nm	yellow	450/45	Brilliant Violet (BV) 515, Pacific Blue, BD Horizon V600
525/50		525/50	BD Horizon V500
610/20		610/20	WAVE, Quanta 605
780/60		780/60	WAVE, Quanta 605
260/60			WAVE, Quanta 605 (optional in place of one of the above filters)
488 nm	blue		
560/20			
635/50			
405 nm	green		
525/50			
610/20			
780/60			
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SPECTRAL FLOW CYTOMETER

“one detector, one marker” paradigm

- 1979 cytometric measurement of FITC+ PI+ cells using a video camera as a detector
- 2004 single cell spectral flow cytometer based on a dispersing element and PMT array
- 2006 spectrograph implemented in the optical pathway of a conventional FC

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SPECTRAL FLOW CYTOMETER

Focus of a Conventional Flow Cytometer

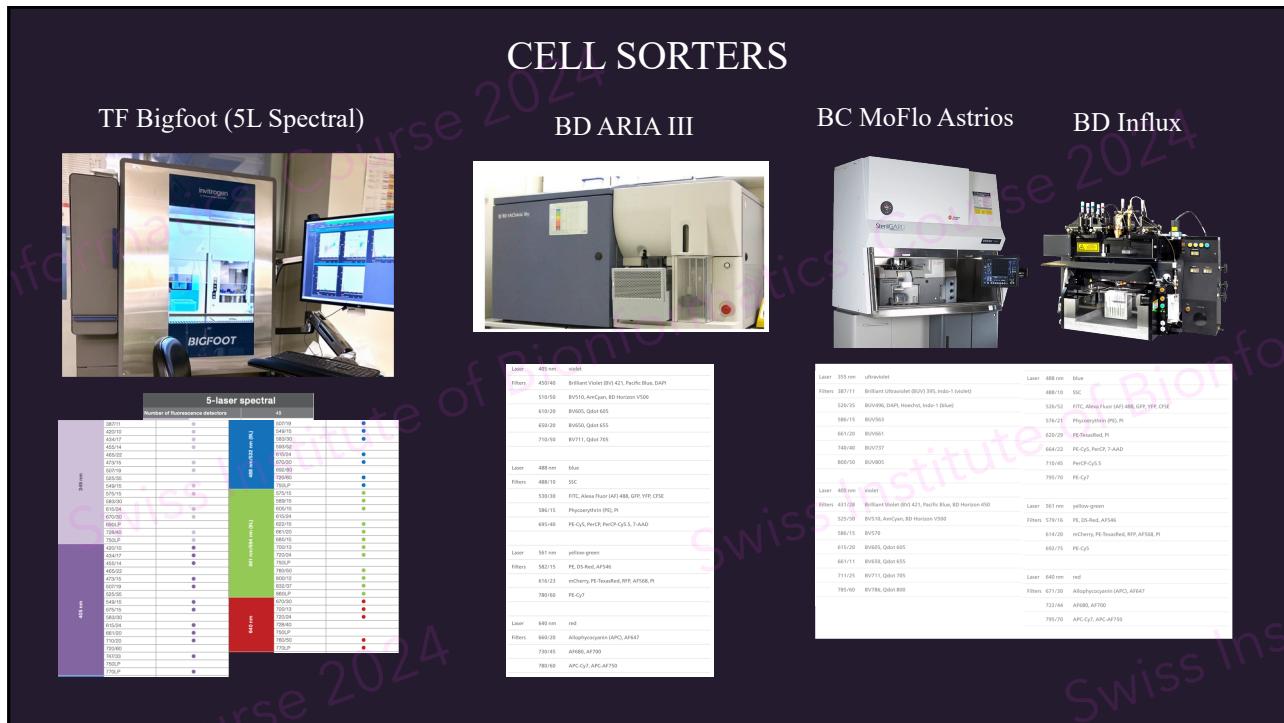
Focus of a Spectral Cytometer

Applications are similar to those performed on conventional FC

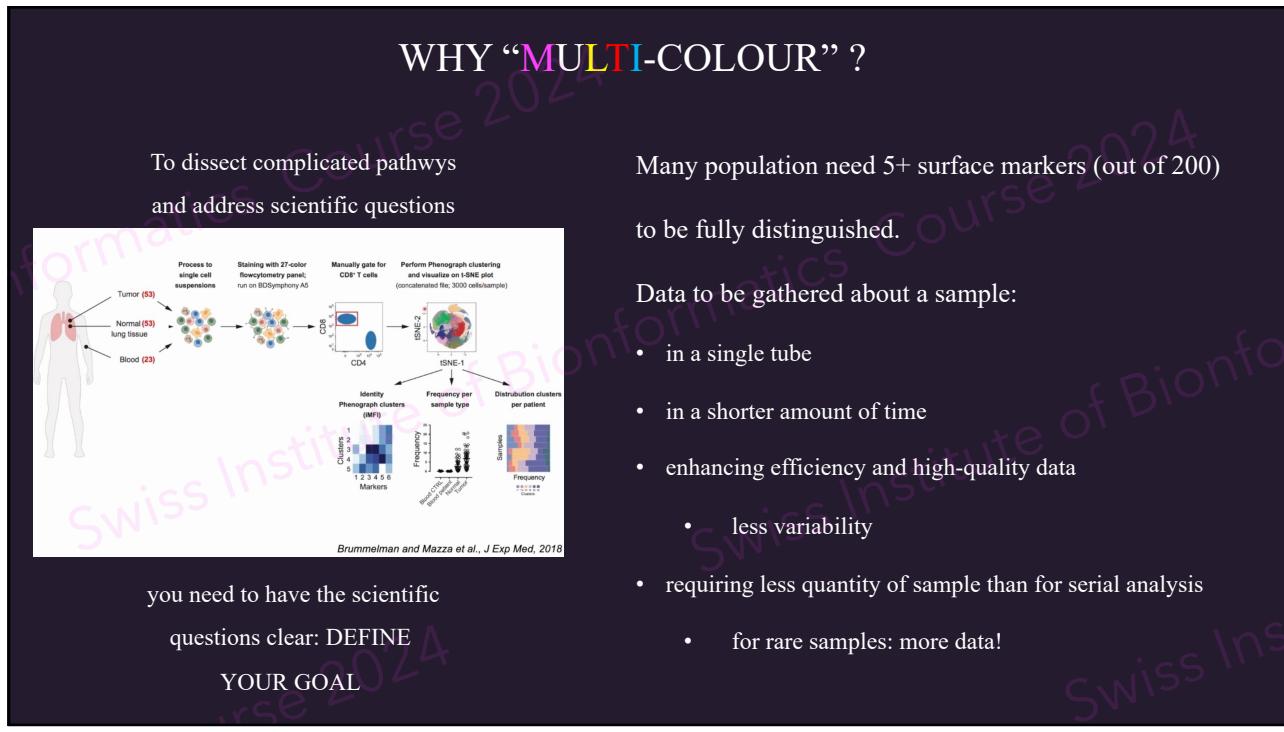
Plus:

1. You can find differences in other parts of the emission spectrum between dyes with the same emission peak = **Allowed to be used simultaneously!**
2. Also the **Autofluorescence** as a signature, so it can be treated as a separate channel and its interference can be cleaned off other channels = **Better resolution!**

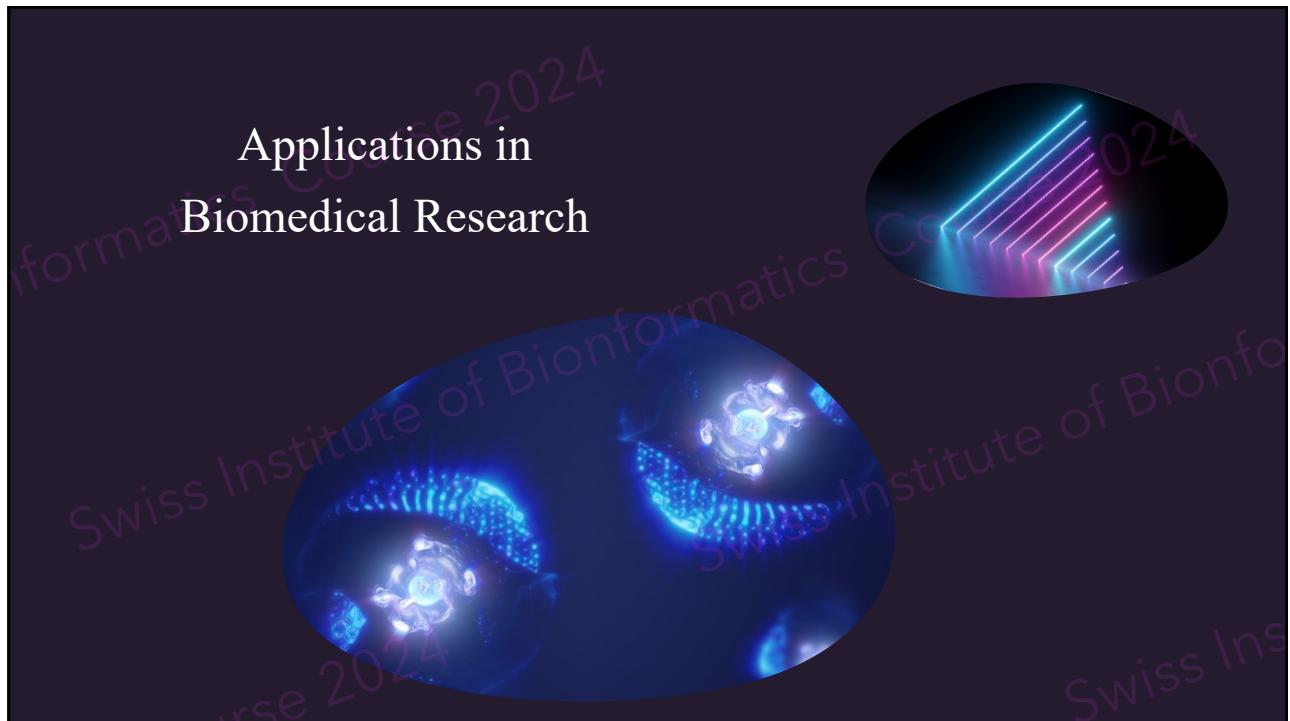
10



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Specific mRNA detection

The systematic modulation of mRNA governs the complicated biological functions of our cells.

DNA microarray and RNA-Seq : unable to measure gene expression at the single cell level (need to pre-sorting)

Correlation between gene expression and protein level is often difficult to demonstrate and requires different platforms and dedicated biological material.

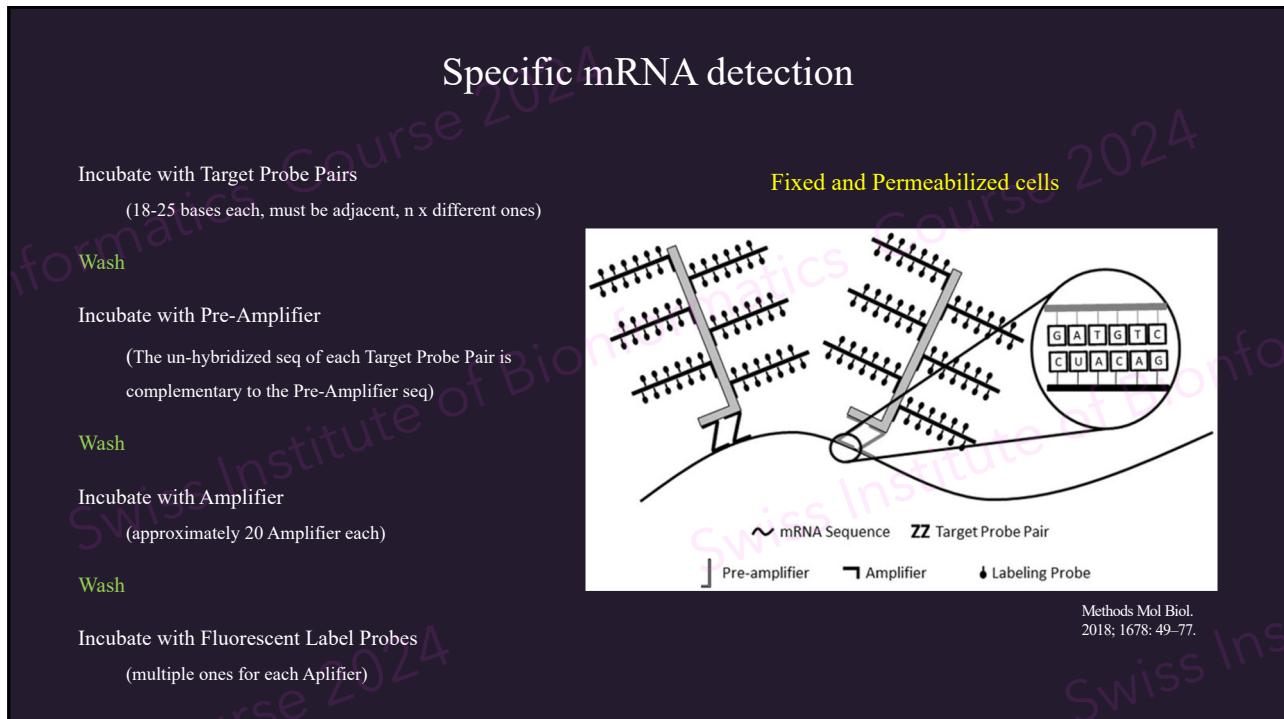
Branched DNA

a signal (not target) amplification assay

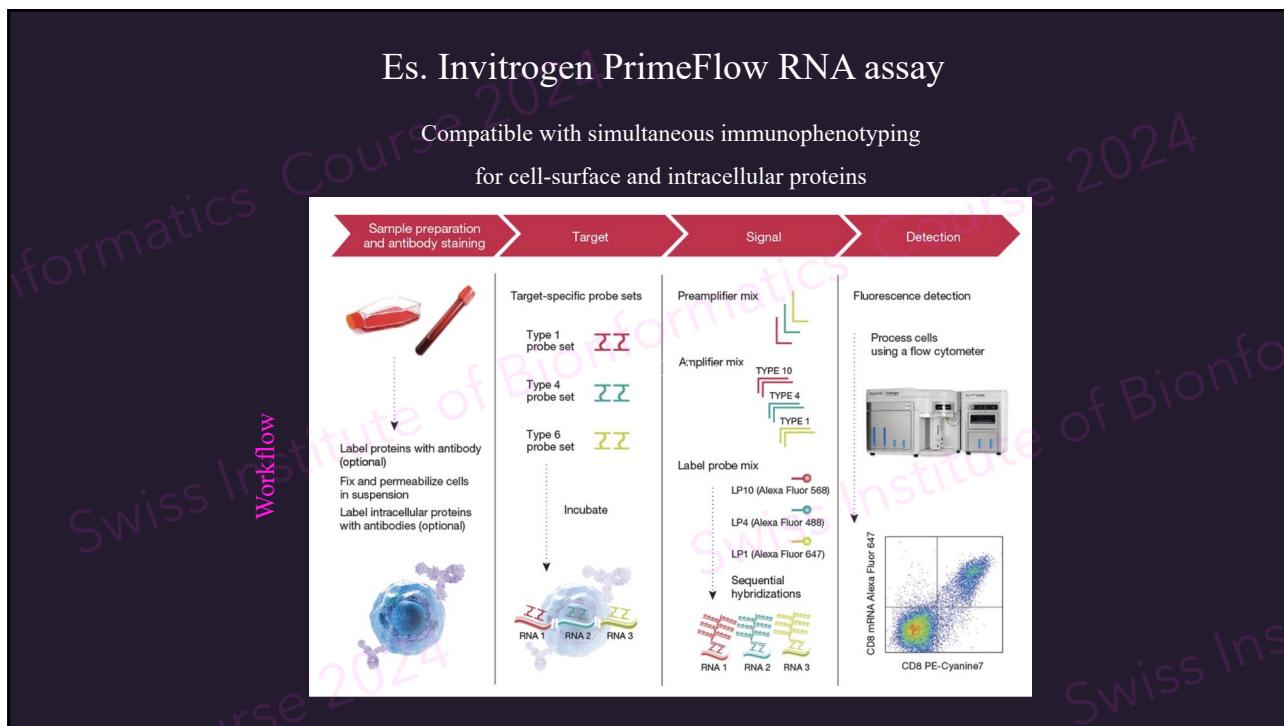
Allows you to

- correlate the measurement between mRNA transcripts and antigen expression
- at single cell level
- to labelling cell targets for which Abs do not exist

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Cell-membrane enzymes activity

Enzymes expressed on the cell-membrane, such as disintegrins, sheddases, metalloproteinases, have key catalytic role on vital cellular functions.

- Substrates: cytokines, growth factors, adhesion molecules
- Human disease implications: cancer, heart disease, diabetes, rheumatoid arthritis, kidney fibrosis and Alzheimer's d.
- Present also on Exosomes surface

To directly measure their association with specific cells and functions:

individual-cell membrane-bound enzyme activity assays

↓

Processing of specifically-bound FPS (FRET Peptide Substrate), Glutaraldehyde (GAL)-crosslinked, with consequent generation of coupled fluorescent products

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Cell-membrane enzyme activity

Cell processing of FPS substrate and the staining with its fluorescent product (that binds to individual-cell membrane) depend on:

time and temperature

concentration & temperature

By setting fixed time/temp/conc. :

measure differences in enzymatic activity and correlate to its relative densitiy of expression (and any other makers)

Sample-1 surface markers gated

Sample-2 surface markers gated

Gorry et al J Cancer. 2020; 11(3): 702-715.

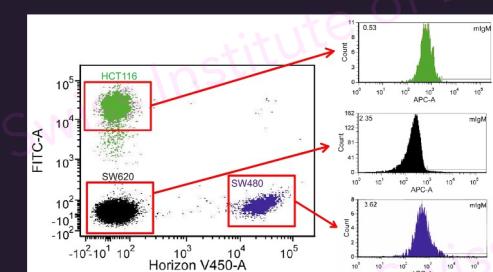
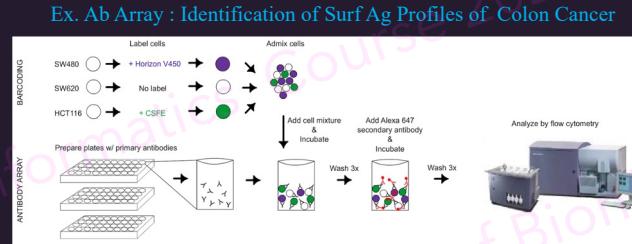
18

Fluorescent Cell Barcoding

Screening experiments, both for drug discovery and basic research in immunology

Barcode / Label individual samples with unique signatures of fluorescence:

- Fluorescent dyes reactive to amine functional groups
 - Covalently attached to the cells (or washed away)
 - Different concentrations of dye identify samples with unique intensity
 - Multiplex samples
 - Stain
 - Analyze as a single sample FACS experiment

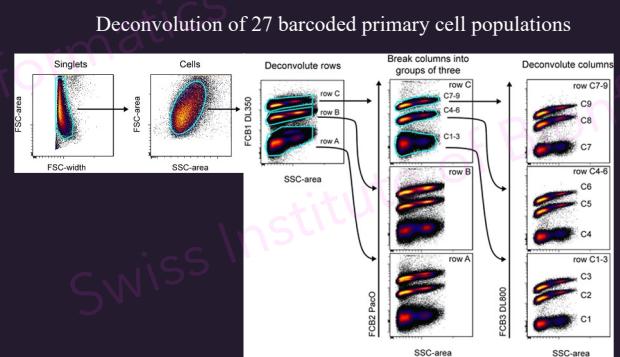
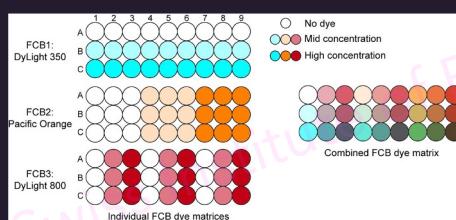


Sukhdeo K et al. PLOS ONE, 2013

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Fluorescent Cell Barcoding

Layout of barcoding matrix used to encode 27 samples



Curr Protoc Cytom, 2011 Jan;Chapter 6:Unit 6.31

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Fluorescent Cell Barcoding

Advantages:

- Reduced Ab consumption 10- to 100-fold
- No required modification of existing antibody staining panels
well-suited for FCB are those parameters normally not utilized for standard stainings
(Pacific Orange, DyLight 800 or 350)
- Increased data robustness
(ctrls and tests combined) minimized pipetting and staining variation, no normalization
- Enhanced speed of acquisition
large profiling, high content FACS experiments

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Cytometric Bead Assay

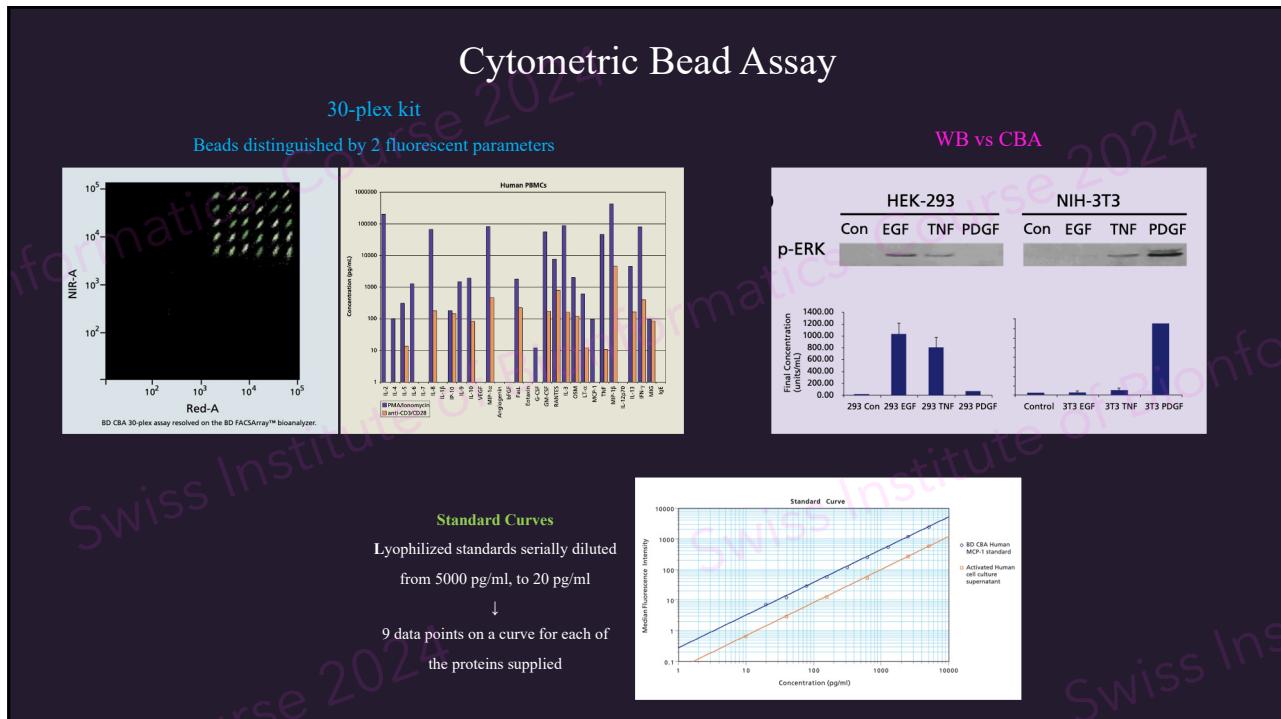
Cytometric Bead Assay (**CBA**) methodology uses fluorescent latex beads coated with **capture** antibody to quantify multiple proteins simultaneously in a single tube by measuring the fluorescence intensity of a fluor-conjugated **detection antibody**.



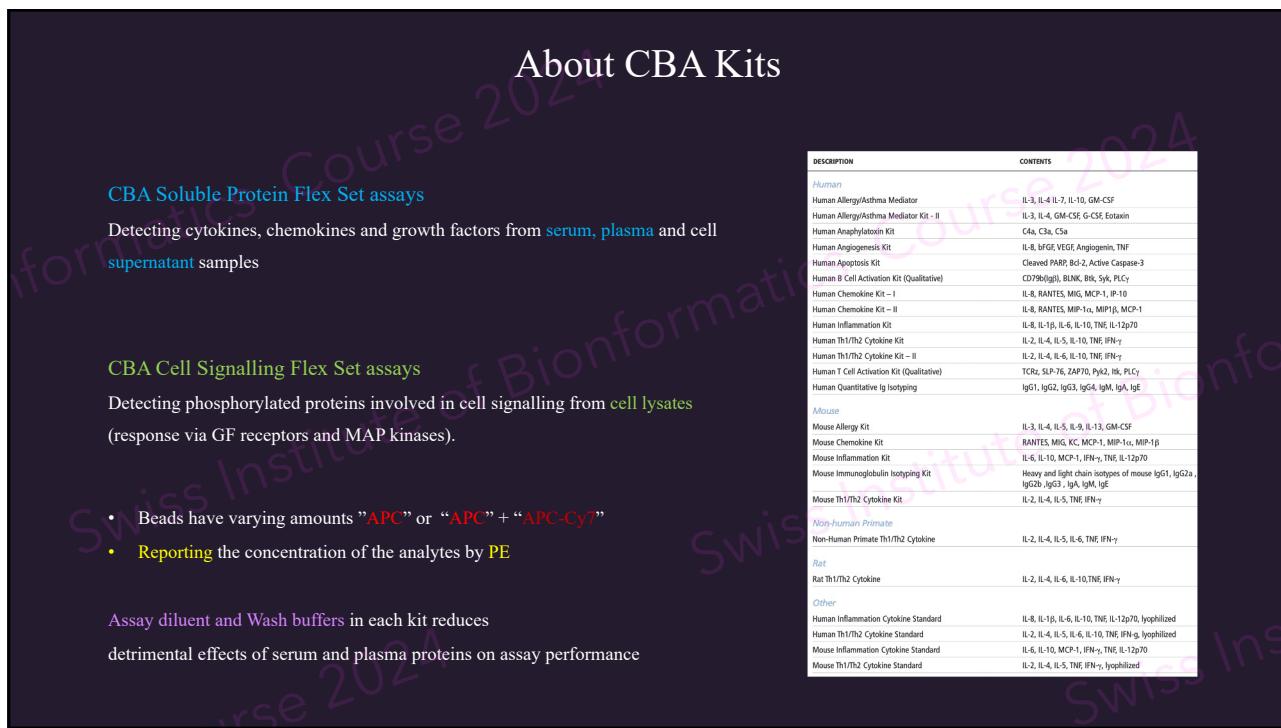
In principle is a fluid-phase “sandwich” assay but it significantly reduces

- sample volumes of analytes
 - time of labour
- compared to the traditional ELISA and Western blot techniques.

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Signalling network analysis

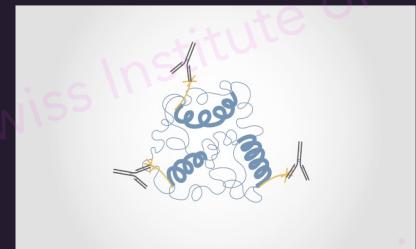
Intracellular signal transduction is primarily mediated by the reversible **phosphorylation** of signalling molecules.

Kinases transfer phosphate groups from ATP to specific serine, threonine or tyrosine residues of target molecules.

Result: altered **activity** level, subcellular **localization** or tertiary **structure**.

Phosphorylation-site analysis provides definitive information on functional relationships between signaling proteins:

- Indicates what protein or pathway might be activated
- Indicates potential drug targets
- Monitor signalling cascade



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Phosphoprotein analysis

FACS analysis is time saving and it requires less cells compared to other techniques (ex. WB)

If you start from frozen samples: let them adapt for 2h before starting any stimulation.

Find the best timing to observe the signal

- Fix the cells precisely then

Time 0 30'' 60'' 90'' 120'' 150'' 2 min 5 min



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Phospho-Flow

Preserve the epitope integrity

- Quick / Strong Fixation
Es. Cold + PFA Hi conc.
- & Permeabilization
Es. 90% Acetone/MetOH
(ice cold)

Check literature, set your own protocol on Neg / Pos ctrls, before using on large scale

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Phospho-Flow

- Run all conditions in one batch
Keep the same environmental conditions
(es. Thermoblock on Unstimulated / Time 0)
- Use always fresh reagents
Fixatives efficiency may be altered after long usage or after expiration date
- Proteins may have multiple phosphorylation sites:
Be sure your Ab recognizes the correct one!

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Cell Senescence

When cells reach their limit of divisions:

Senescent phase

- Metabolically active
- No more replications
- No apoptosis
- Multi-nucleated (cancer senescence)
- Senescence-associated vacuoles (SAVs) with intense proteolytic activity in the peripheral cytoplasm
- pH-dependent β -galactosidase
- Altered mitochondria

Role in tumor suppression, tumor progression, aging, and tissue repair.

The diagram illustrates a senescent cell with several characteristic features:

- CELL CYCLE ARREST**: Indicated by a vertical line with a break.
- SASP** (Secreted Senescence-Associated Secretion): Represented by red dots.
- DECREASE OF LAMIN B1 CONTENT**: Indicated by a decrease in the amount of lamin B1.
- INCREASED LYSSOMAL MASS**: Indicated by a large pink circle containing yellow dots.
- p21 \uparrow** and **p16 \uparrow** : Indicated by green and blue arrows pointing to the cell cycle arrest point.
- S β GAL**: Indicated by a pink circle.
- ENLARGED AND DAMAGED MITOCHONDRIA**: Indicated by a purple circle.
- DNA DAMAGE FOCI DDR ACTIVATION**: Indicated by a pink circle.

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Lysosomes analysis

There is no single good marker for determining senescence, so these cells are identified through the collective interpretation of multiple markers.

β -galactosidase activity

resides in lysosomes
 β -galactoses into monosaccharides under acidic pH
Optimal pH 4, test usually pH6
2x activity independently of test pH

A graph showing two curves: Absorbance (left y-axis, 350-650 nm) and Fluorescence emission (right y-axis, 350-650 nm). The absorbance curve has a peak at approximately 400 nm. The fluorescence emission curve has a peak at approximately 520 nm, shifted to the right of the absorbance peak.

Fluorescent substrate following β -galactosidase hydrolysis:

CellEvent Senescence Green Probe

Resuspend in 1X PBS
Fix in 4% PFA, 10 min at RT
Stain with Senescence Probe:
90 min, 37°C (no CO₂)
Wash
Resuspend
Acquire

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Mitochondria

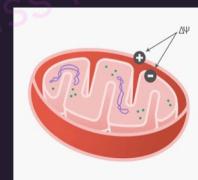
- 10% of the cell volume
- structural variations depending on cell type, cell-cycle stage and intracellular metabolic state
- energy production through oxidative phosphorylation (OxPhos) and lipid oxidation
- Steroid biogenesis, intracellular Ca^{2+} homeostasis, apoptosis

Mitochondrial quality analysis

- Not only senescence
- Reactive oxygen species production influences immune responses against bacteria
- Center of many innate immune responses & cancer
- Dysfunction regulates the immunopathology of various diseases
- Metabolism studies

• Damaged:

- Don't generate the same level of energy
- Accumulate in/out triggering innate immune response
- Depolarized membrane (+ Hi ROS)



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Mitochondria analysis (Mito-Flow)

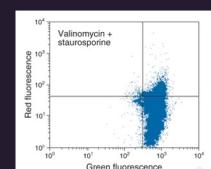
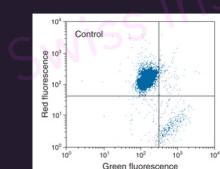
Potential

Mitochondrial Membrane Potential Probe T3168 /
JC-1

- Positively charged → accumulate in the electronegative interior of the mitochondrion.
- Low concentrations = low membrane potential: monomers, E_m 529 nm.
- High concentrations = High potentials: internal aggregates, E_m ~590 nm
- Depolarization is indicated by a decrease in the red/green fluorescence intensity ratio
- Not fixable

MitoTracker Orange, Red, Deep Red

- Tetramethylrosamine / X-rosamine derived probes
- Potential-dependent accumulation
- aldehyde-based fixatives + permeabilization with cold acetone do not disturb the staining pattern



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Mitochondria analysis

<p>Mass</p> <p>MitoTracker Green</p> <ul style="list-style-type: none"> • cell-permeant mitochondrion-selective dyes • potential-independent dye • decreased intensity → degradation of mitochondria (mitophagy) • colocalization with autophagosome / lysosome corresponding marker (Imaging Flow Cytometry) <p>CellLight Protein-based</p> <ul style="list-style-type: none"> • GFP or RFP reporter genes + leader sequence of E1α pyruvate dehydrogenase 	<p>Oxidation</p> <p>Orange CM-H₂TMRos Red CM-H₂XRos</p> <ul style="list-style-type: none"> • Reduced forms of the tetramethylrosamine / X-rosamine (MitoTrackers) • do not fluoresce until they enter an actively respiring cell, <ul style="list-style-type: none"> ◦ oxidized to the fluorescent form ◦ sequestered in the mitochondria <p>The diagram illustrates the chemical transformation of CM-H₂TMRos into a fluorescent form. It starts with the non-fluorescent CM-H₂TMRos derivative, which has a quinone-like structure with two methylamino groups (N(CH₃)₂) at the 4 and 4' positions. An arrow labeled "Oxidation" points to the CMTMRos derivative, where one of the methylamino groups is oxidized to a highly fluorescent cationic form (N⁺(CH₃)₂). A second arrow labeled "Thio-conjugation" points to the final fluorescent conjugate, where the CMTMRos derivative is linked via a thioether bond (-CH₂S-) to a peptide chain.</p>
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Reactive Oxygen Species

Role of reactive oxygen species (ROS) **in metabolism and stress response** in microbiology and infectious diseases

ROS production in response to antibiotic exposures

Several methods for detecting ROS, including electrochemistry, spectroscopy, chemiluminescence:

Never **single-cell resolution** of endogenous ROS

Reactive fluorescent dyes

Study of ROS in intracellular environment, high-content and high-throughput analyses.

- Hydroxyphenyl fluorescein (HPF)
- chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H₂DCFDA)
- CellROX Green dye

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Reactive Oxygen Species

The CellROX™ Green

Cell-permeable and very weakly fluorescent while in the reduced state
Upon oxidation: binds DNA, Em 508/525 nm, remains to the nucleus and cytoplasm

- Simple protocol
- N-acetyl cysteine (antioxidant, negative control)
- tert-butyl hydroperoxide solution (TBHP, an inducer of ROS)

Zebrafish

Stem Cell Reports Vol. 8 j 360–372, 2017

www.thermofisher.com

Human cell line

Bacteria gating Workflow

Front. Microbiol., 21 March 2017 | <https://doi.org/10.3389/fmicb.2017.00459>

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Met – Flow

Single-Cell Metabolic Analysis

Mitochondria analysis
✓
ROS analysis
✓

Cells utilize metabolic pathways to coordinate function-specific gene expression at the epigenetic, transcriptional, post-transcriptional, and post- translational levels

Lack of ability to interrogate these pathways at single-cell level within a heterogeneous population

↓

Met-Flow is a high-parameter flow cytometry method utilizing **antibodies against metabolic proteins** that are critical and **rate-limiting** in their representative pathways

Met-Flow **does not directly measure flux** of a pathway

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Met – Flow

Ten metabolic proteins were chosen and optimized based on their critical role in specific metabolic pathways

Table 1 Metabolic proteins representing critical components and rate-limiting enzymes of metabolic pathways.				
Gene name	Gene description	Metabolic pathway	Function (HPA, RefSeq)	Localization (HPA)
SLC20A1/ PiT1	Solute carrier family 20 member 1	Phosphate transporter	Sodium-dependent phosphate import, regulates ATP^+ , has role in LPS-induced inflammation ⁹¹ , and in CD4^+ -mediated ⁹² Rate-limiting step of urea cycle, alters p53- AKT signaling ⁹³ , regulates nitric oxide generation ⁹⁴ , maintain arginine levels ^{95,96}	Vesicles
ASS1	Arginosuccinate synthase 1	Arginine biosynthesis	Rate-limiting step of urea cycle, alters p53- AKT signaling ⁹³ , regulates nitric oxide generation ⁹⁴ , maintain arginine levels ^{95,96}	Cytosol, nucleoplasm
SLC2A1/ GLUT1	Solute carrier family 2 member 1/ facilitated glucose transporter member 1	Glucose uptake	Glucose import, responds to hypoxia and glucose starvation, influenced by mTOR activity ⁹⁷	Plasma membrane
IDH2	Isocitrate dehydrogenase (NADR(+)) 2	TCA cycle	NAD ⁺ -dependent, NAPDH producing role in energy production ⁹⁸ , maintains glutathione and peroxiredoxin systems ^{99,100}	Mitochondria
G6PD	Glucose-6-phosphate dehydrogenase	Oxidative PPP	Rate-limiting step of oxidative PPP ^{101,102} , provides NADPH and pentose phosphates for fatty acid and nucleic acid synthesis ¹⁰³	Cytosol, MTOC, vesicles
ACAC/ ACC1	Acetyl-CoA carboxylase alpha	Fatty-acid synthesis	Rate-limiting step in de novo long chain fatty-acid synthesis ^{103,104}	Cytosol, nucleoli, fibrillar center
PRDX2	Peroxiredoxin 2	Antioxidant	Peroxiredoxin family of antioxidant enzymes, prevents oxidative stress ^{105,106}	Cytosol
HK1	Hexokinase 1	Glycolysis	Rate-limiting step in the first stop of glucose metabolism, couples glycolysis to intramitochondrial OXPHOS ^{99,100}	Mitochondria
CPT1A	Carnitine palmitoyl-transferase 1A	Fatty-acid oxidation	Key enzyme in carnitine-dependent transport across the mitochondrial inner membrane ^{107,108}	Outer mitochondrial membrane
ATPSA/ ATPSA1/ ATPSFA	ATP synthase F1 subunit alpha	ATP biosynthesis	Catalyses ATP synthesis, ATP-binding soluble catalytic core, F ₁ -transport ^{109,110}	Mitochondria

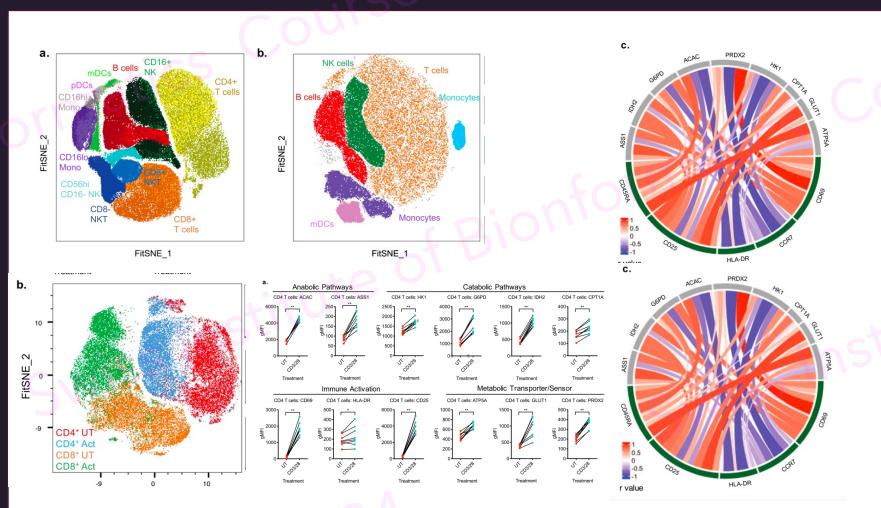
Metabolic proteins and their function are based on transcriptomic and proteomic analysis reported in the Human Protein Atlas (HPA)^{110,111} and by Reference sequence database at NCBI (RefSeq)¹⁰⁷ and published studies.

localized to the mitochondria, the cell surface or the cytosol

In addition, antibodies to surface and intracellular markers were used to phenotype 11 leukocyte subsets in PBMCs to generate a 27 colors flow cytometry panel

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Met – Flow



Protein-level analysis shows divergent metabolic profiles in leukocytes

Activation induces extensive metabolic reprogramming

Hypoxia

Deficiency of Oxygen in cells and tissues, correlated with several pathologies, including cancer and ischemia

Pimonidazole EF5	require cell fixation followed by immunostaining difficult diffusion and require fixation and immunostaining	Mainly in Microscopy
BioTracker	detection of hypoxia in living cells	

Mechanism

Reductive cleavage of the azo-base in the BioTracker occurs under hypoxic conditions generating 2Me RG which emits bright green fluorescence. Lower oxygen levels (more severe hypoxic conditions) lead to greater fluorescence intensities.

www.sigmaldrich.com

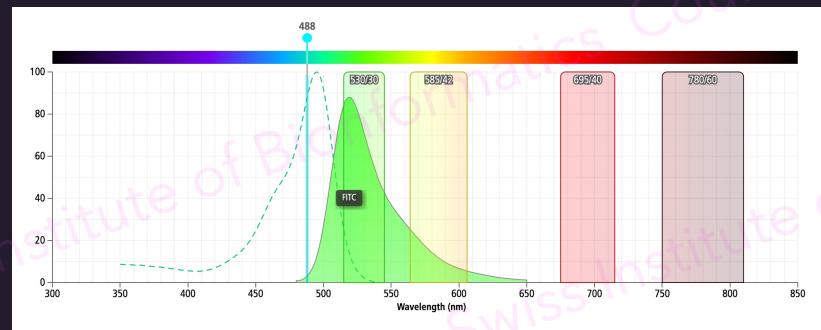
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OBSTACLES along the road

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EMISSION PROFILE

Every fluorochrome has its own and specific excitation and emission spectrum



In this simple 4 colours example there are several overlapping emissions in the band-pass filters, equivalent to a background in that detectors

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THE PRESENCE OF ONE FLUOROPHORE EMISSION IN THE DETECTOR OF ANOTHER IS ALMOST INEVITABLE IN MULTICOLOR FLOW CYTOMETRY

THE PRESENCE OF ONE FLUOROPHORE EMISSION IN ANOTHER'S DETECTORS IS INEVITABLE IN MULTICOLOR FLOW CYTOMETRY

WE CAN DEAL WITH IT:
COMPENSATION

WE CAN DEAL WITH IT:
UNMIXING

#1 source of scientists' frustration and cause of bad data

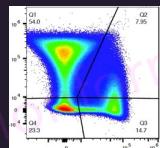
42

SPILLOVER SPREADING ERROR

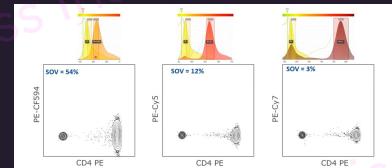
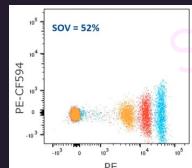
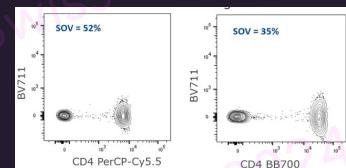
Refers to an error that is visible after compensation or spectral unmixing has been applied

Properly compensated data may not appear linear because of **measurement errors**.

- Low energy of photons (far-red)
- Brighter fluorophores
- Higher density Ag



- High dimensional data
- ↑ the more a fluorophore spills into another's detector

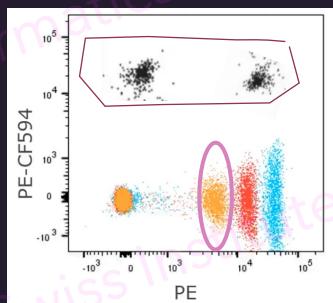


#2 source of scientists' frustration and cause of bad data

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HOW to ADDRESS the SPREAD?

Panel Building STRATEGY



Assigning PE to low expressed Ag

Assigning PE-CF594 to high expressed Ag

Assigning them to not co-expressed Ag

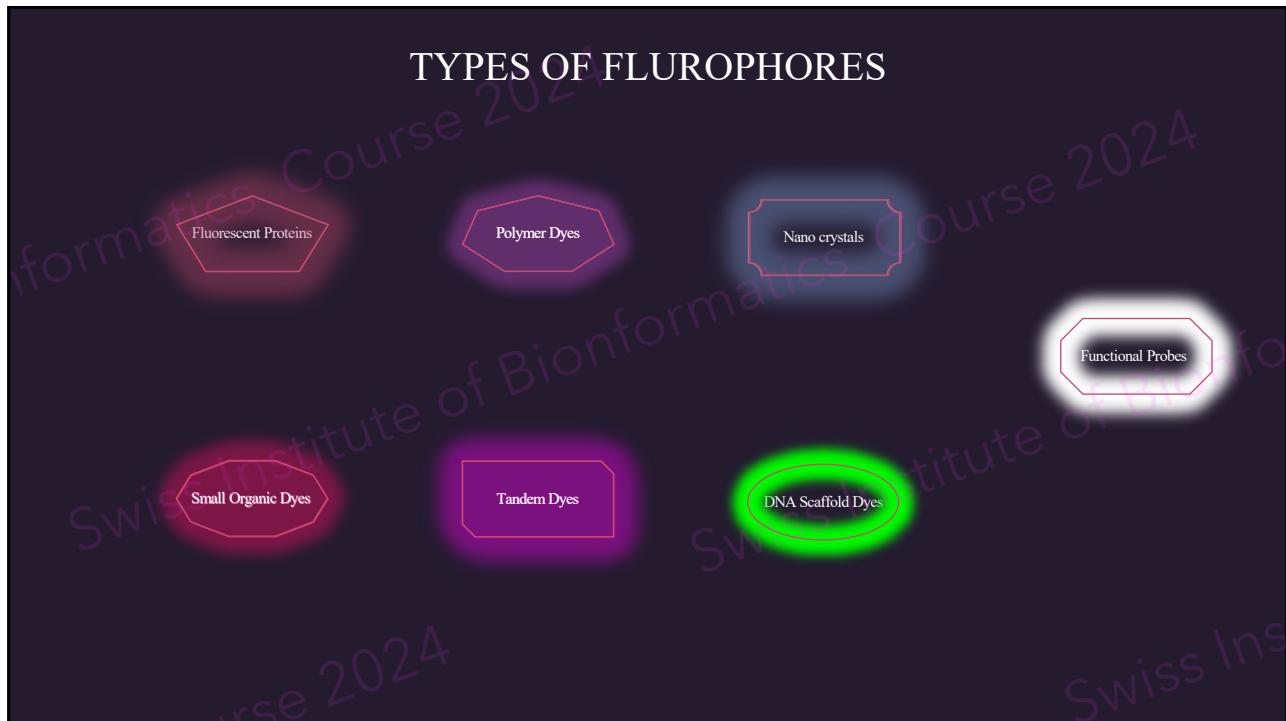
TO DO THAT

KNOWLEDGE REQUIRED

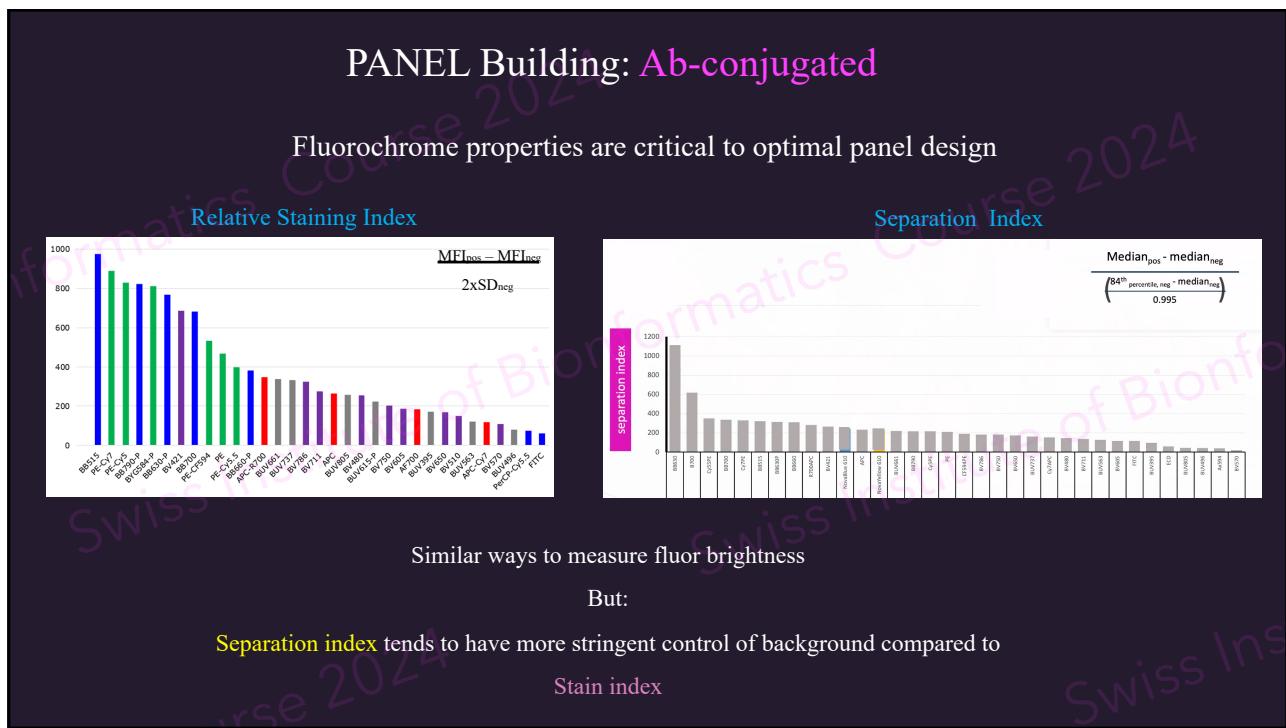
ABOUT ANTIGENS
(& cell population)

ABOUT FLUOROPHORES

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MAXIMIZE THE RESOLUTION

Fluorochrome assignment based on **type** of antigens

- Segregate bright dyes by allocating them on different lineages
- Dim fluorochrome to **Frequent Ag**
- Bright fluorochrome to **Rare Ag**

Antigen density

- Assign lineages to dyes not spreading on key detection channels
- Dim fluorochrome to High density Ag
- Bright fluorochrome to Low density Ag

Co-expression

- Avoid having dyes difficult to resolve (Hi spillover/ similar signature) in a co-expression scenario

Spillover Spread

- Choose the right concentration (**titrate first**)
- Minimal impact in your panel (concentration independent)

Primary: CD3, CD4, CD8
Secondary: CD45RA, CCR7, CD27, HLA-DR
Tertiary: PD-1, CD25, TCR γ/δ

Fluorochrome Brightness

Level of Antigen Expression

CD4, CD127, CD25

Mahnke and Roederer, Clin Lab Med, 2007

```

graph TD
    A[MARKER A] --> B{Are they co-expressed?}
    B -- NO --> C[Understand Why?]
    B -- YES --> D{Balance Expression?}
    D --> E[Choose Reagents]
    E --> F[For A use a dim fluorochrome]
    E --> G[For B use a dim fluorochrome]
    F --> H[Test Your Panel]
    G --> H
    H --> I{Do you get good resolution?}
    I -- NO --> C
    I -- YES --> J[SUCCESS]
  
```

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From Theory to Reality: Commercial AVAILABILITY

we can improve our guidelines as follows

1. Tertiary antigens
 - a) Assess reagent availability (often not too many options available)
 - b) Assign brightest fluorochrome available (use fluorochrome brightness ranking)

2. Secondary antigens
 - a) Based on co-expression of antigens expressed at intermediate levels
 - b) If no co-expression, use any bright dye still available
 - c) If co-expression:
 - Use a bright dye that does not spread into selected fluorochrome for tertiary antigens
 - If only available dyes have spread, use a dim dye to minimize spread impact

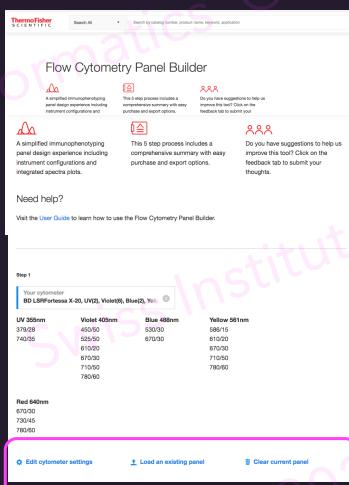
3. Primary antigens
 - a) Often available in many colors
 - b) Assign to dyes that are dim and that have minimal spread in other dyes (examples: FITC, Pacific Blue, BV510, Alexa Fluor 532, APC-H7)

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PANEL BUILDERS

<https://www.thermofisher.com/order/panel-builder>

<https://app.fluorofinder.com>



The screenshot shows the Thermo Fisher Flow Cytometry Panel Builder. At the top, there's a search bar and a link to the product specification sheet. Below it, a section titled "Flow Cytometry Panel Builder" provides a brief overview of the process. A table lists available lasers and their wavelengths:

Laser	Wavelength
UV Laser	350nm
Violet Laser	405nm
Blue Laser	488nm
Yellow Laser	561nm
Red Laser	640nm
Green Laser	532nm
Pink Laser	594nm
White Light	785nm

Below this, there are three main sections: "Add antibodies and reagents", "Fluorescent proteins", and "Antibodies you need". Each section has dropdown menus for target species and specific products. To the right, there are four separate panels, each showing a histogram for a different laser wavelength (UV, Violet, Blue, Yellow) with a red peak indicating the selected product.

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NEW TOOLS

Easy Panel Platform

The screenshot displays several windows from the Easy Panel Platform:

- Login Screen:** Shows fields for Email and Password, and a "Create an account?" link.
- Advanced Panel Builder:** A dashboard for building panels. It includes sections for "Panel Overview", "Panel Components", and "Panel Requirements".
- Panel Editor:** A tool for creating panels. It shows "Panel Structure" and "Panel Components".
- Panel Details:** A window showing panel details for "AntiA Flu700 (AP7700, AlexaFlu700)". It includes sections for "Select Antigen", "Select Expression Level", "Select species reactivity", and "Select Fluorochrome".
- Panel Requirements:** A window showing requirements for "AntiA Flu700 (AP7700, AlexaFlu700)". It includes sections for "Select Antigen", "Select Expression Level", "Select species reactivity", and "Select Fluorochrome".
- Panel Screenshot:** A heatmap titled "Panel Similarity Matrix" showing similarity scores between various panel components.
- Panel Selection:** A window titled "Panel Selection" showing a list of panels with their names and descriptions.
- Panel Comparison:** A window titled "Panel Comparison" showing a heatmap comparing two panels.
- Panel Report:** A window titled "Panel Report" showing a detailed report for a selected panel.
- Panel Summary:** A window titled "Panel Summary" showing a summary of the selected panel.

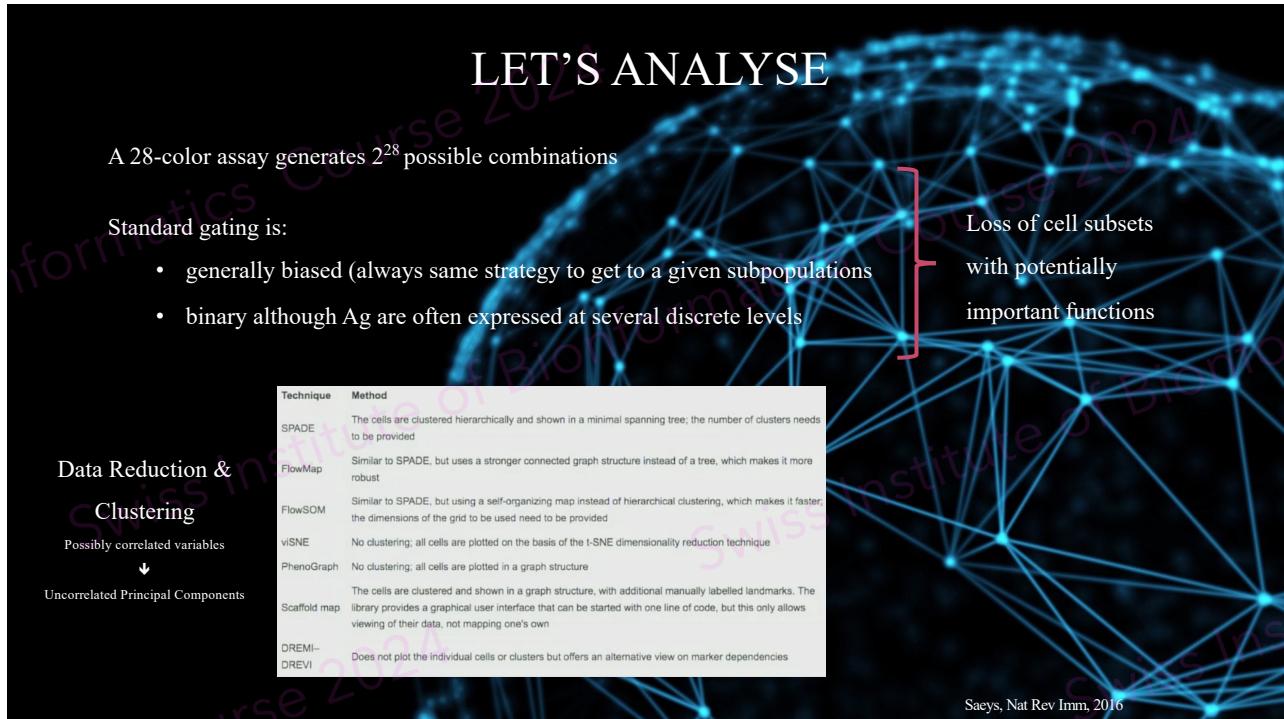
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Is this the right way?
Anyone getting lost?

The image block contains three circular icons:

- Group looking at a map:** A group of people in a forest looking at a physical map.
- Compass:** A close-up of a traditional compass rose.
- GPS Device:** A close-up of a GPS navigation screen showing a map and route information.

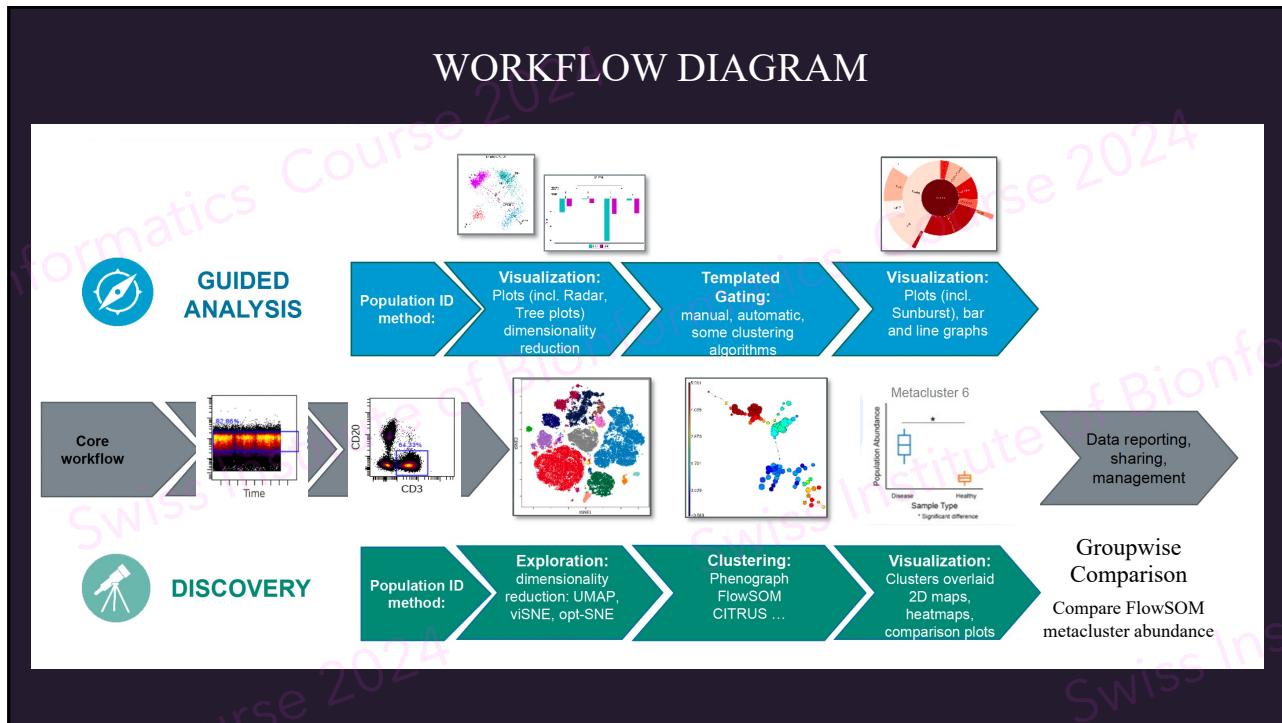
52



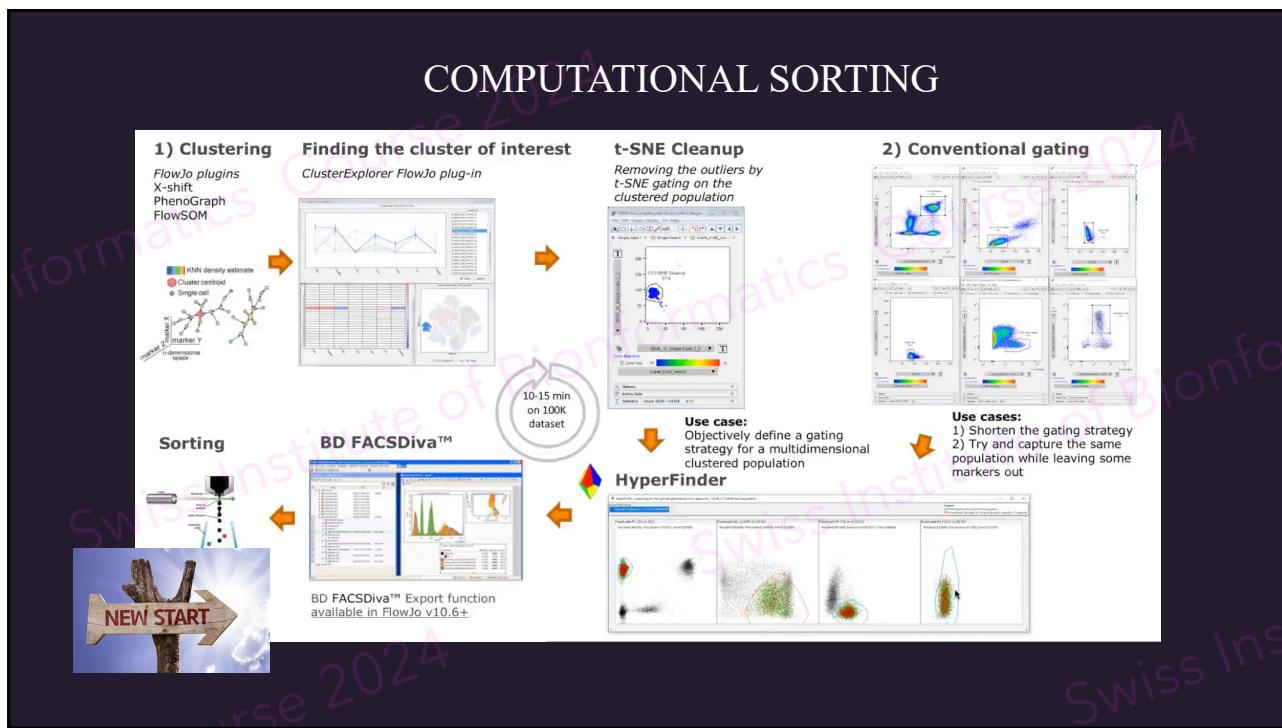
53



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