SH800 Cell Sorter Training

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Day 1:

- Presentation: Basics of sorting with the SH800

Day 2:

- Maintenance & Troubleshooting
- Hands-on practice with samples

SH800 System Overview + Accessories

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- **Easy-to-use cell sorter!**
  - Automated Sort Setup
  - Intuitive Software
- **Sorting Chip**
  - 70/100/130 µm nozzle
  - Replaceable sample path: sorting chip + sample line
- **Configurable Optics**
  - 4 lasers: 405, 488, 561, 638 nm
  - 6 colours + 2 scatters
- **Sorting options**
  - 2-way sorting
  - Tubes & Plates (96 & 384)
  - Index Sorting
- **Biosafety Cabinet**



Setup Beads

Sorting Chips

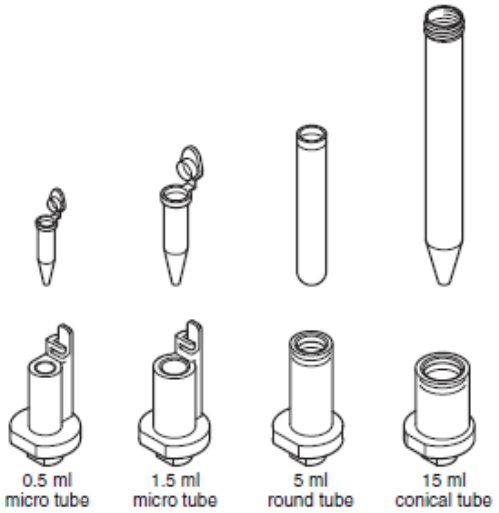


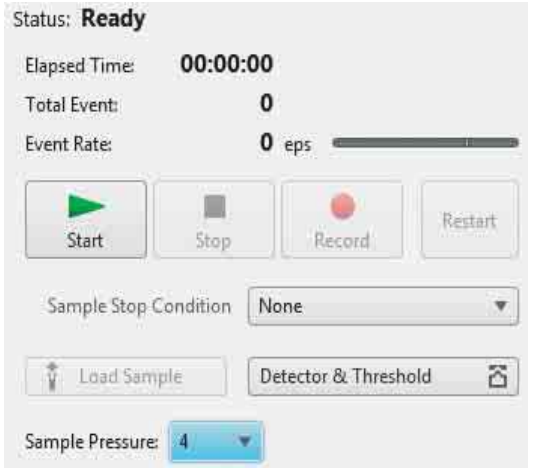


Exchangeable PEEK Sample Line

SH800 – Flexible Sampling Design



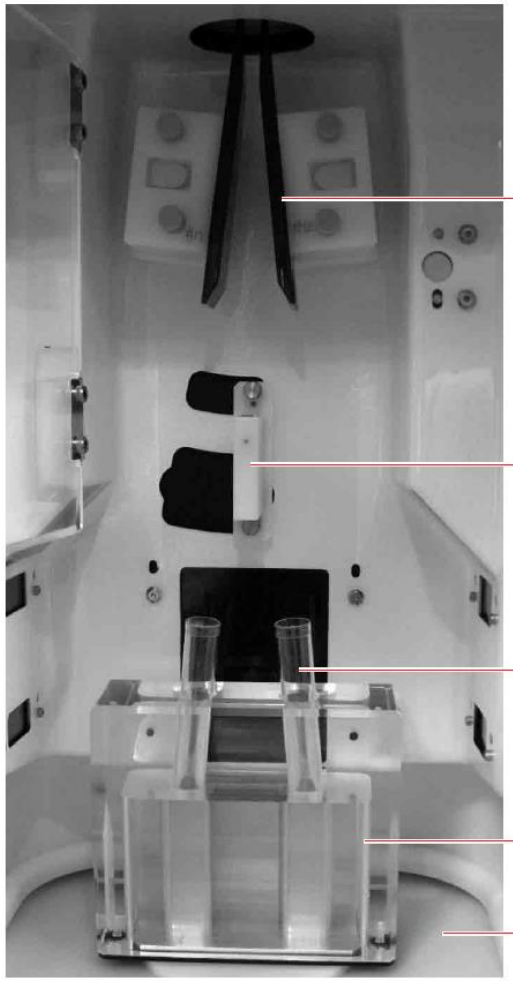




Adjustable sample pressure

- Various sample tube sizes
- Automatic load and positioning
- Sampling in sealed compartment
- Onboard sample agitation (always active)
- Sample temperature control

SH800 Sorting Options

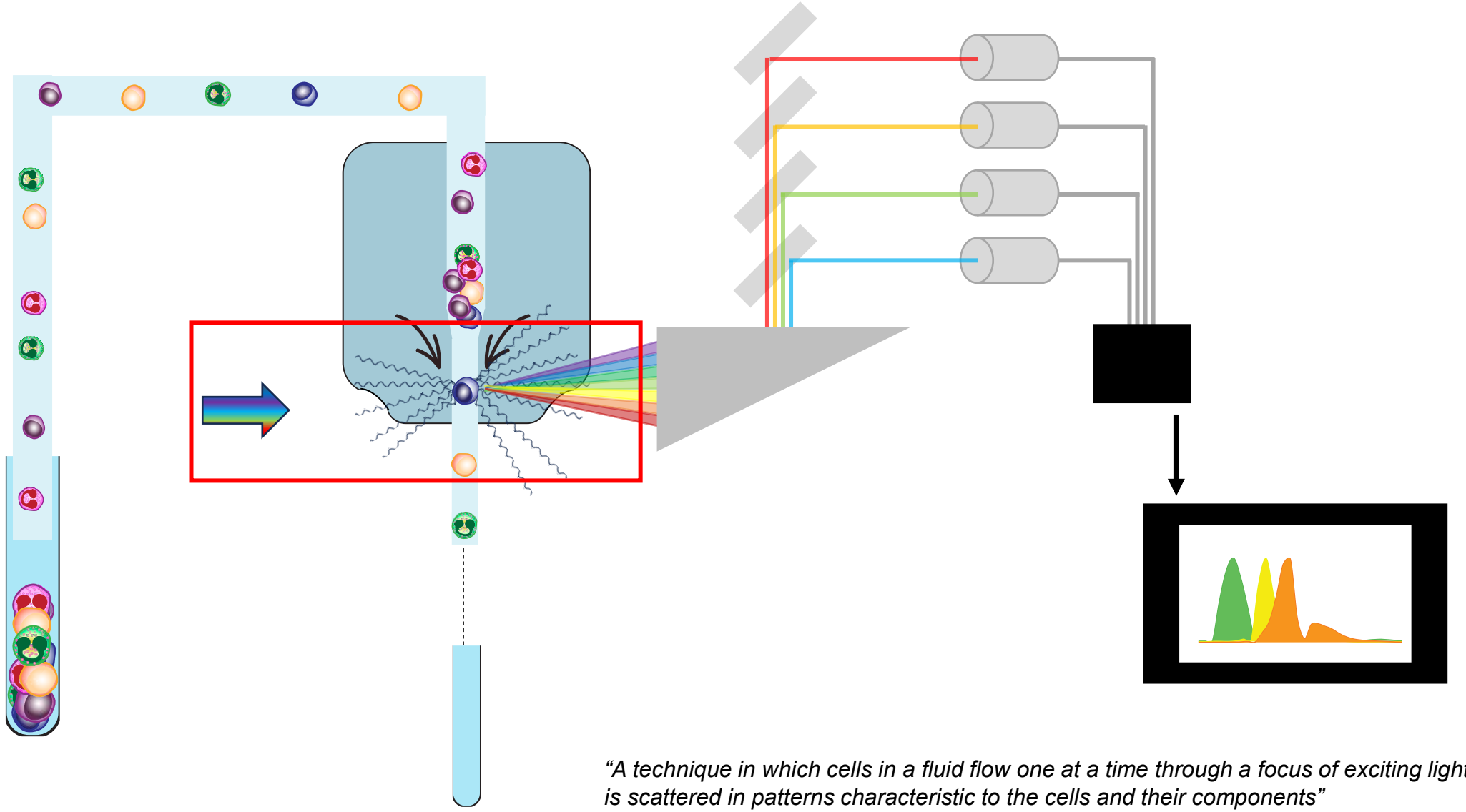


- 2 way tube sorting
- Collection tube temperature control



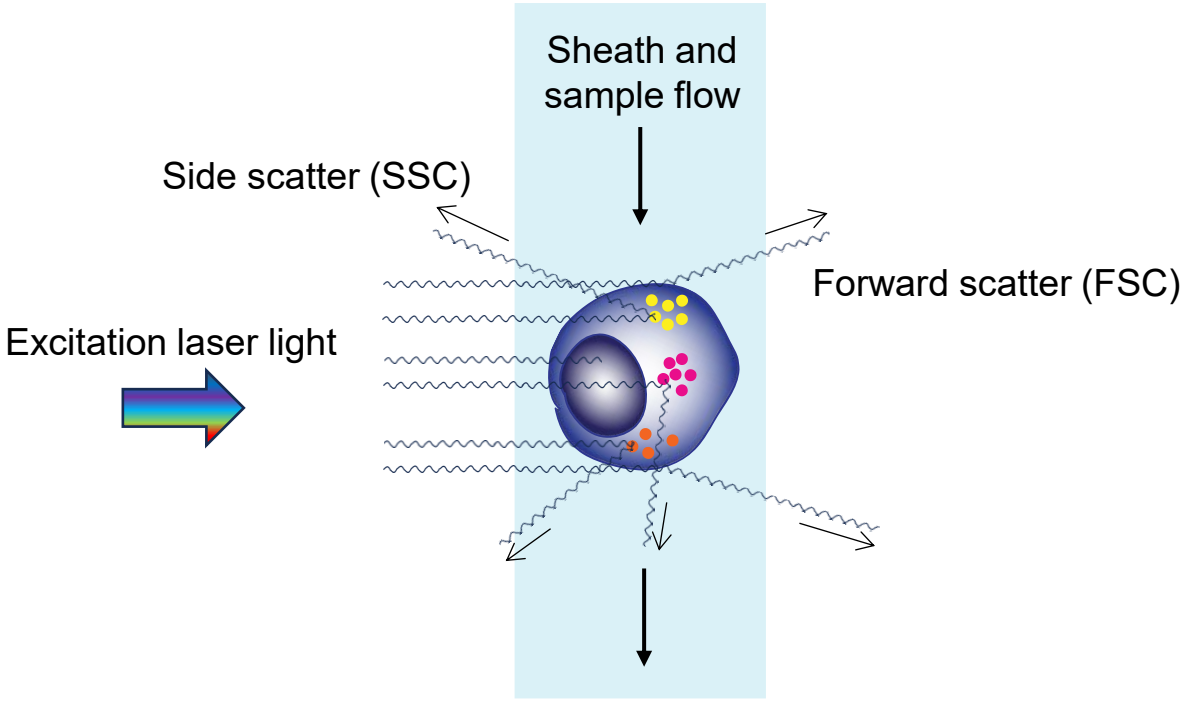
- Plate sorting: TC and PCR plates
- 6, 12, 24, 48, 96, 384-well plate

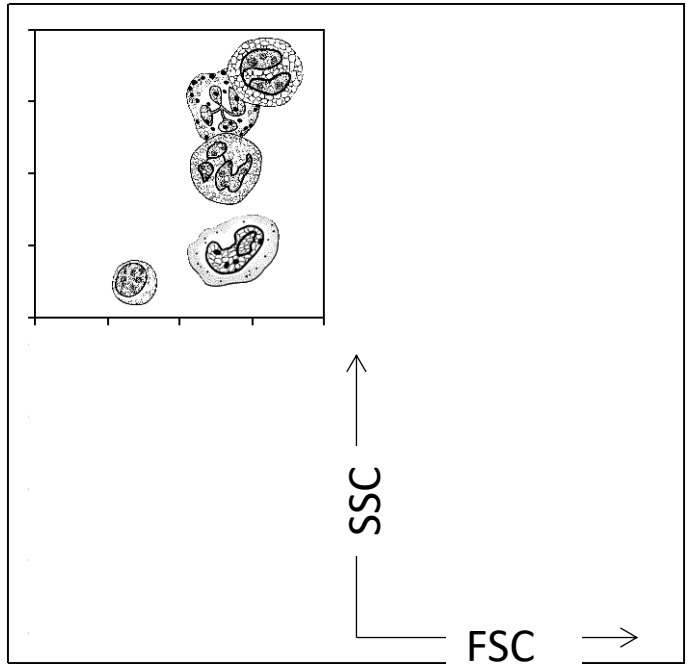
Basic principles of flow cytometry



“A technique in which cells in a fluid flow one at a time through a focus of exciting light which is scattered in patterns characteristic to the cells and their components”

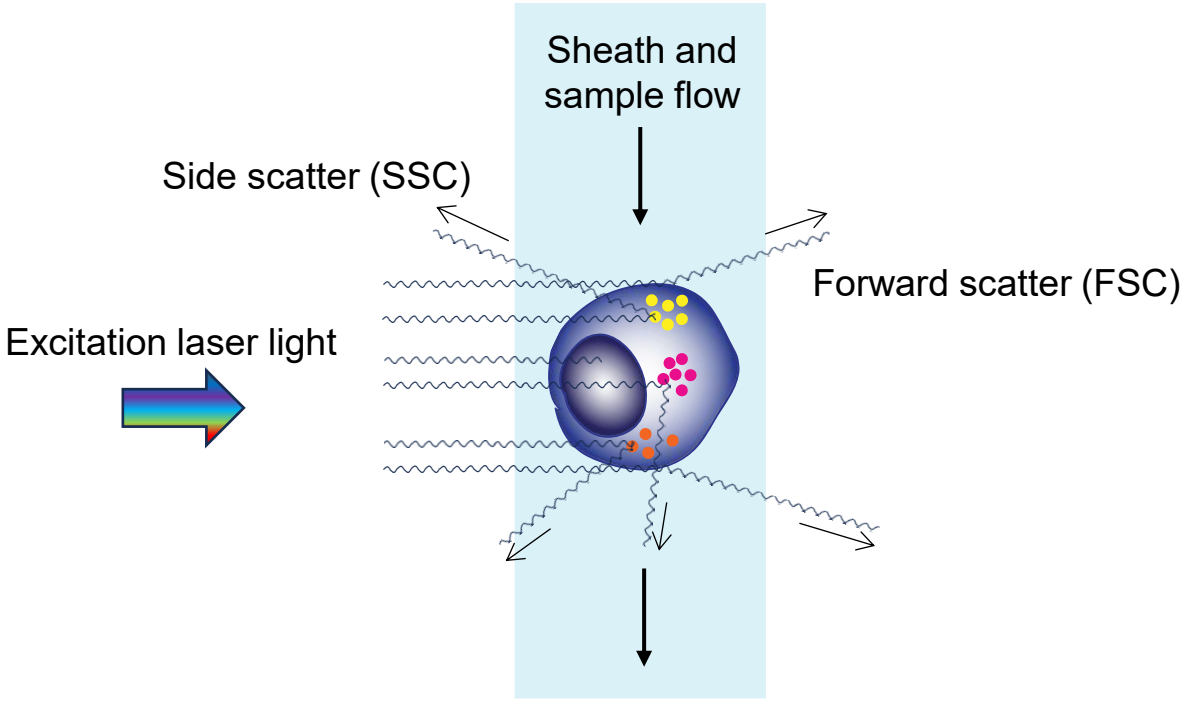
Basic principles of flow cytometry

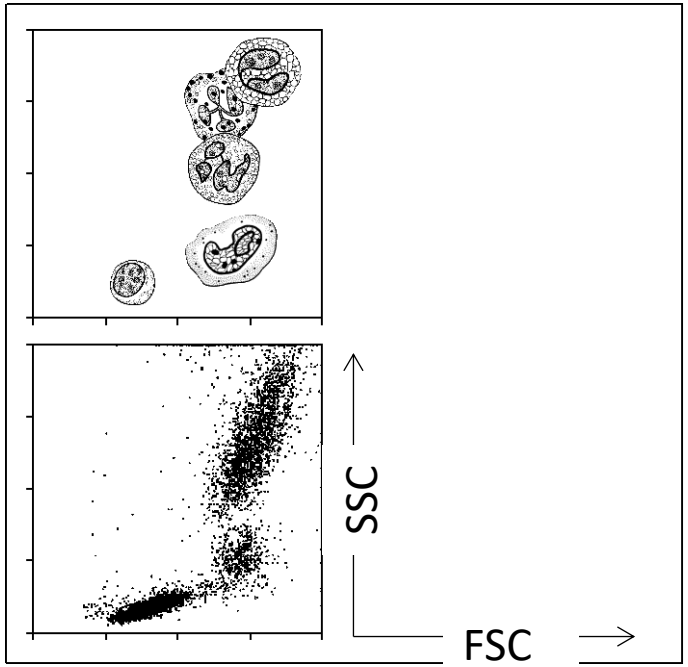




Forward Scatter (FSC): Diffraction of light relative to cell size.
Side Scatter (SSC): Refraction and reflection of light relative to intracellular complexity.

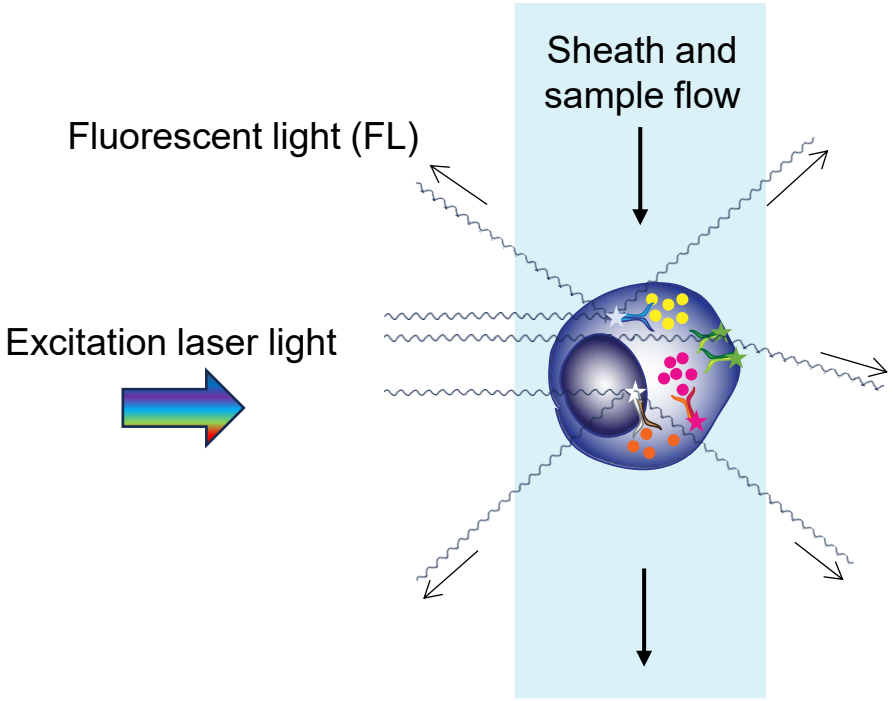
Basic principles of flow cytometry

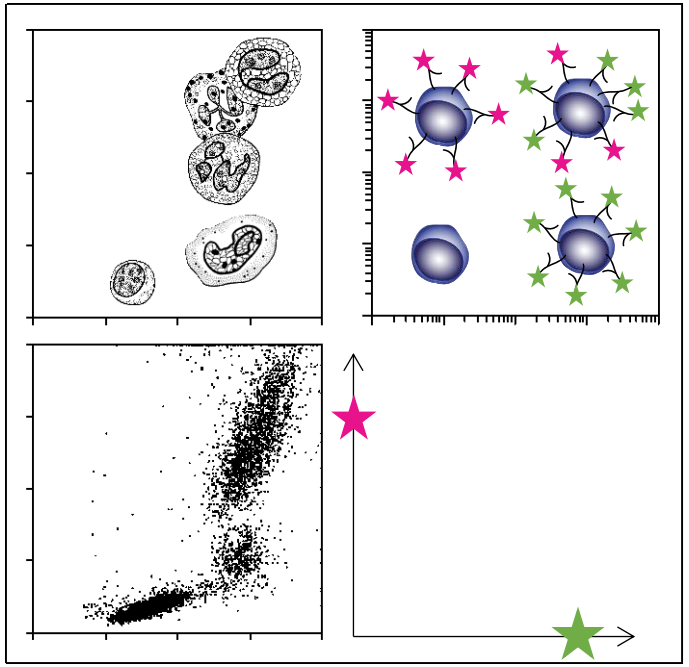




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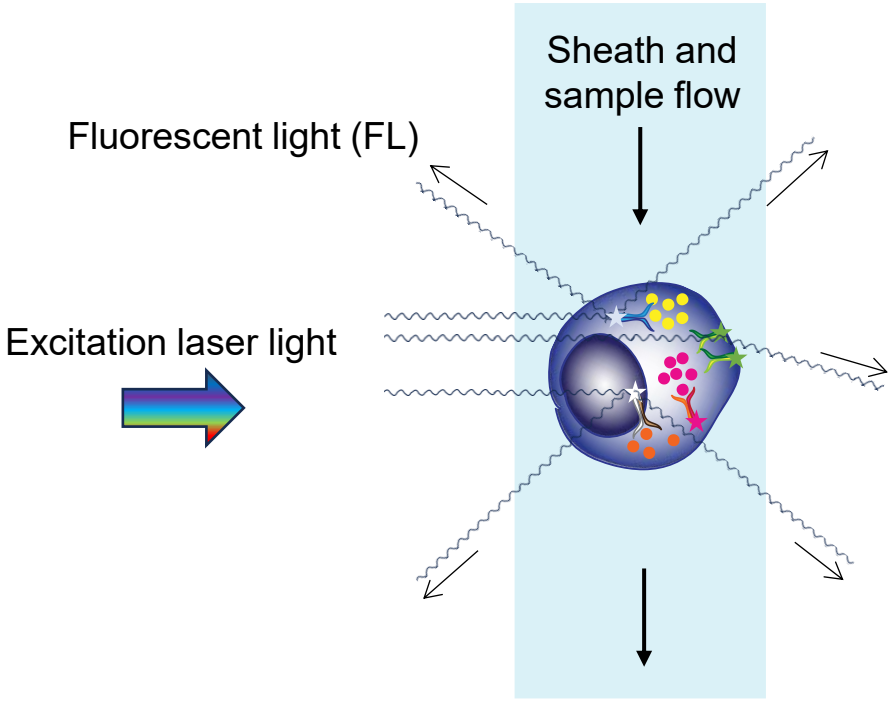
Basic principles of flow cytometry

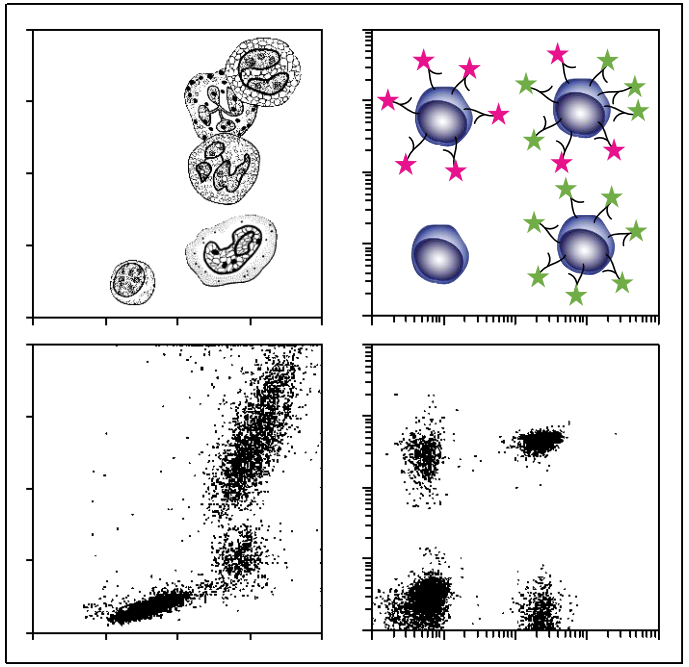




Forward Scatter (FSC): Diffraction of light relative to cell size.
Side Scatter (SSC): Refraction and reflection of light relative to intracellular complexity.
Fluorescence: Fluorochrome-labelled antibodies or intrinsic fluorescence.

Basic principles of flow cytometry

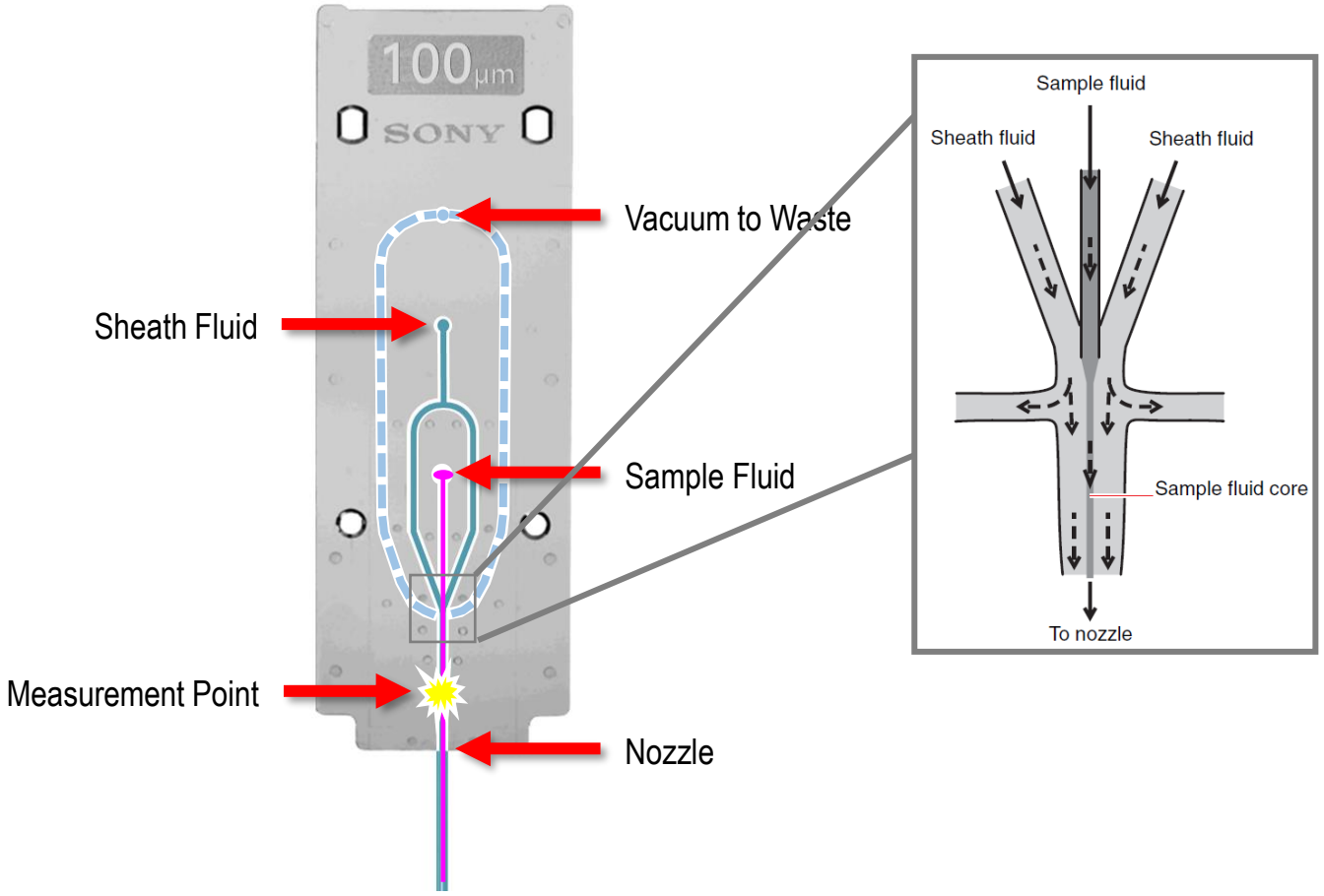




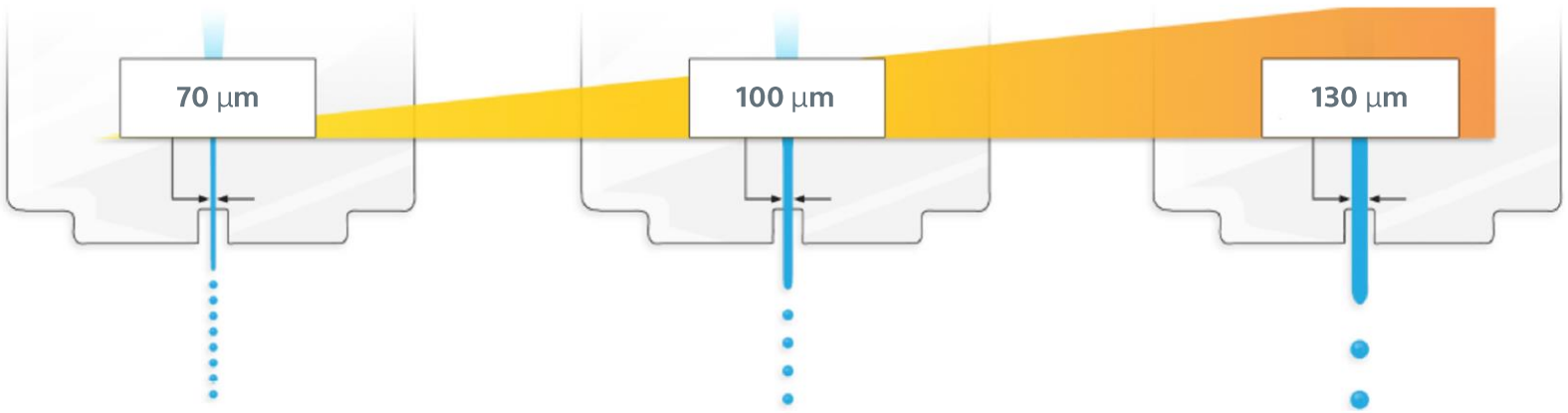
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Microfluidics Sorting Chip

Sample cell diameter should be 5-7x smaller than nozzle diameter!



Microfluidics Sorting Chip



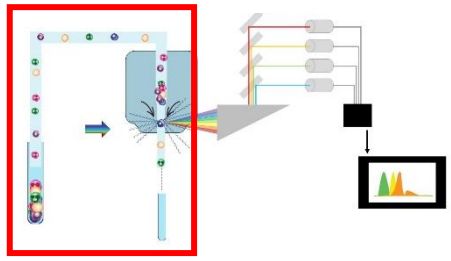
- Fastest sorting
- For small cells only (< 10 µm diameter)

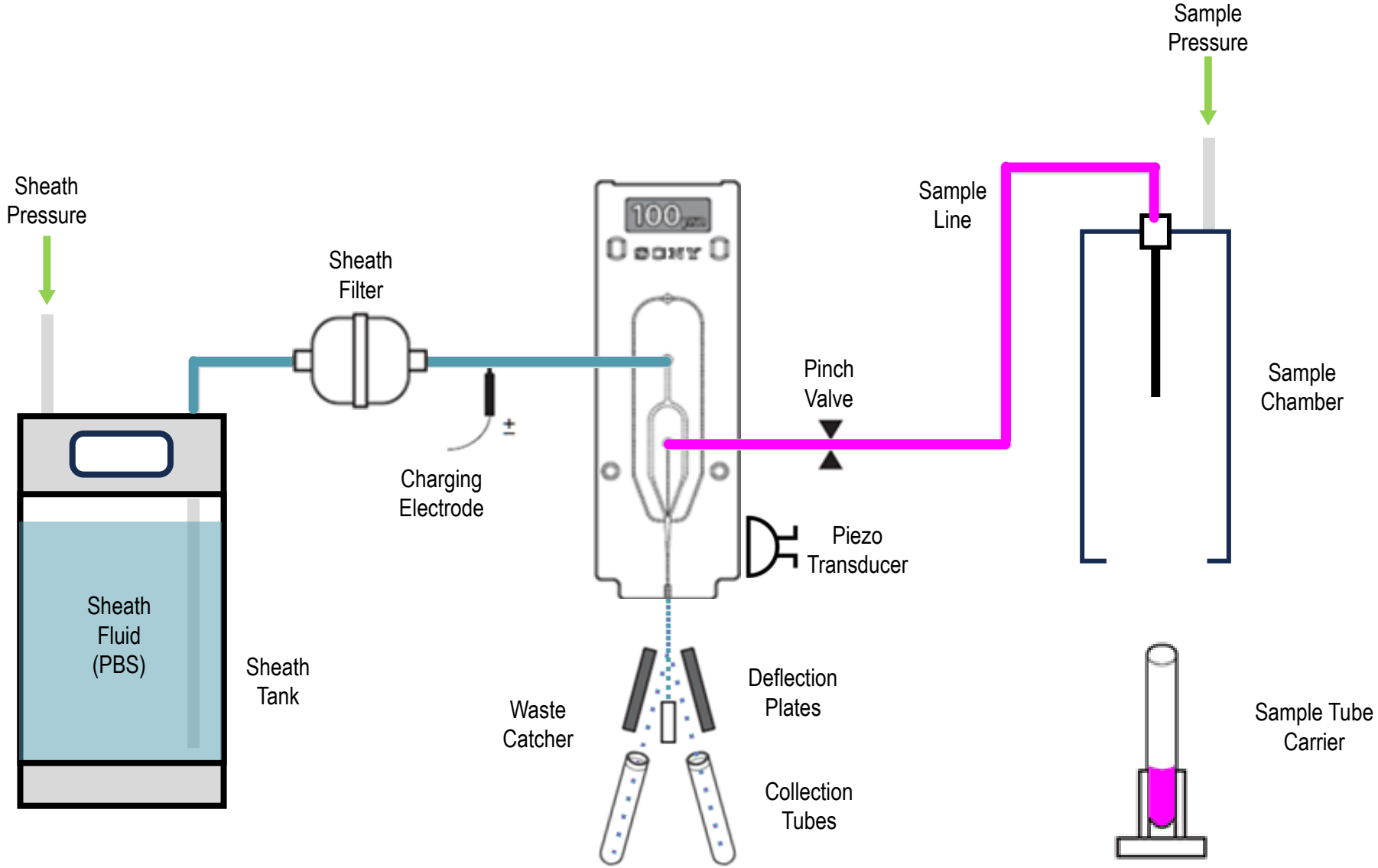
- Used for common cell types < 20 µm in size

- Best for viability of large/fragile cells
- Slowest sorting

Bacteria	Yeast	PBMCs
RBC	Lymphocytes	Cell lines
Splenocytes		Stem cells
Macrophages		Large fragile cells
		Adherent cells
		Epithelial cells
		HEK293T

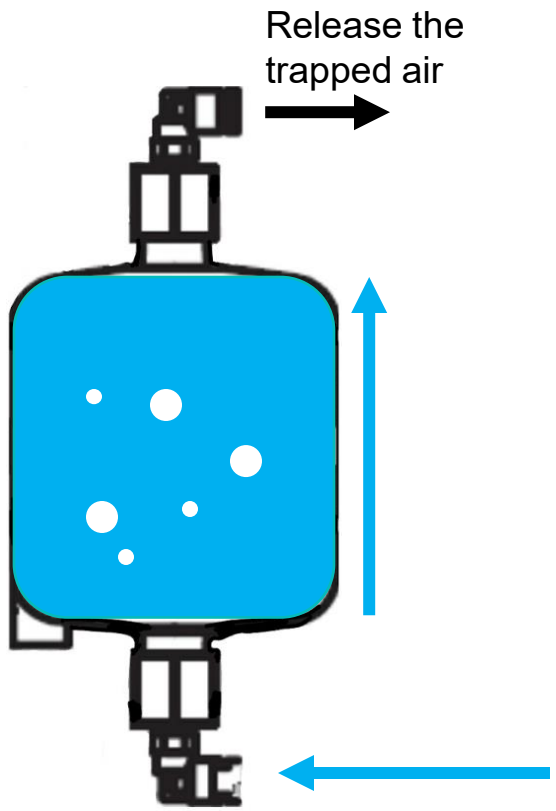
Fluidics System





Start-up

- Sheath tank full
- Waste tank empty
- Remove the collection device
- De-bubble the sheath filter

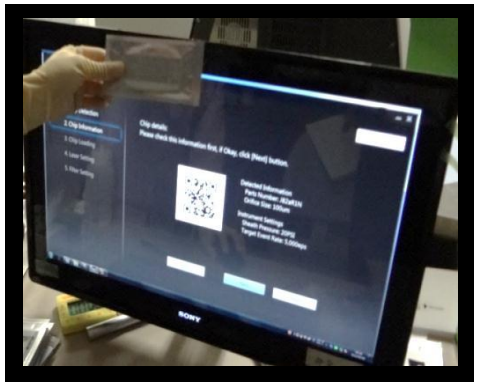


System setup

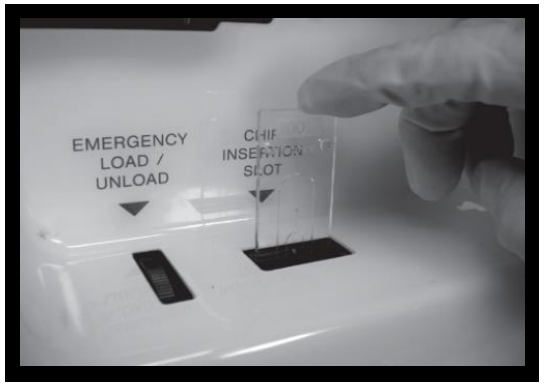
- Set up the system in two steps:
  1. Insert the chip + run fluidics check (priming and de-bubbling)



- Serialised
- Individually sealed
- Clean room packaging



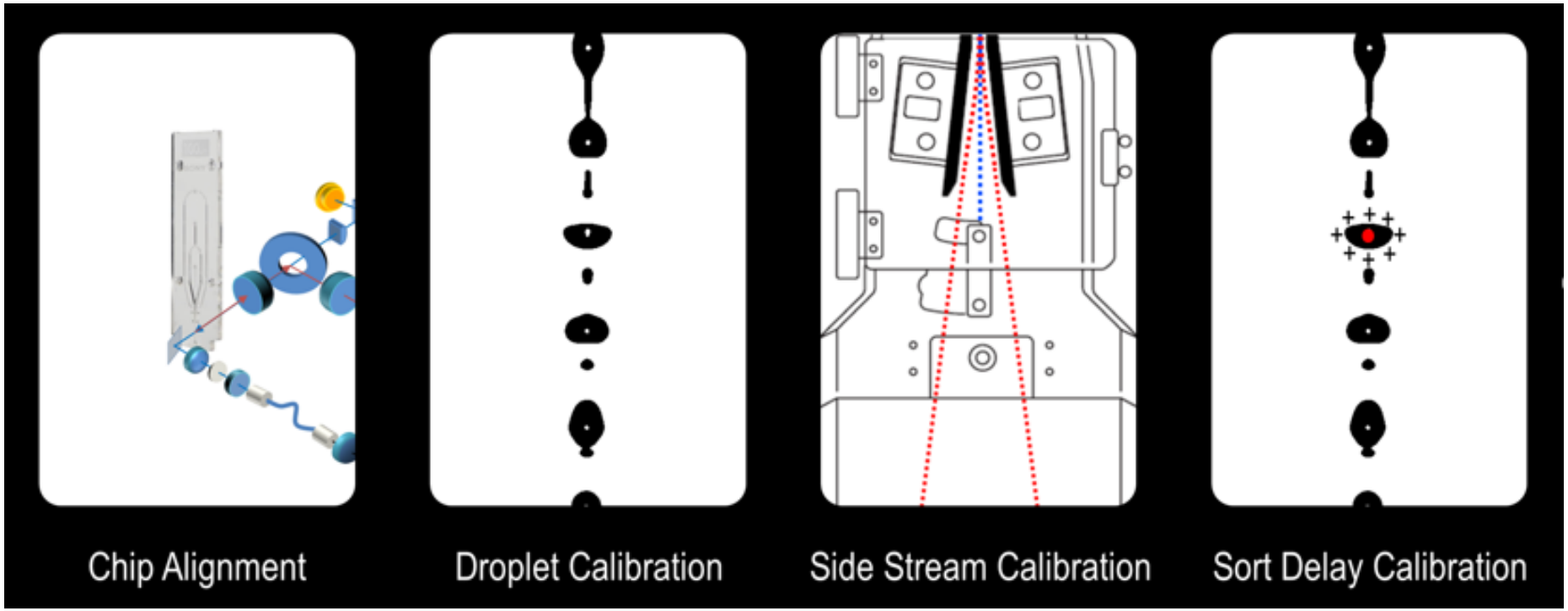
- QR code scan
- Includes chip size and optimal fluidics settings



- One-touch install
- Every day a fresh start

  2. Autocalibration

Autocalibration

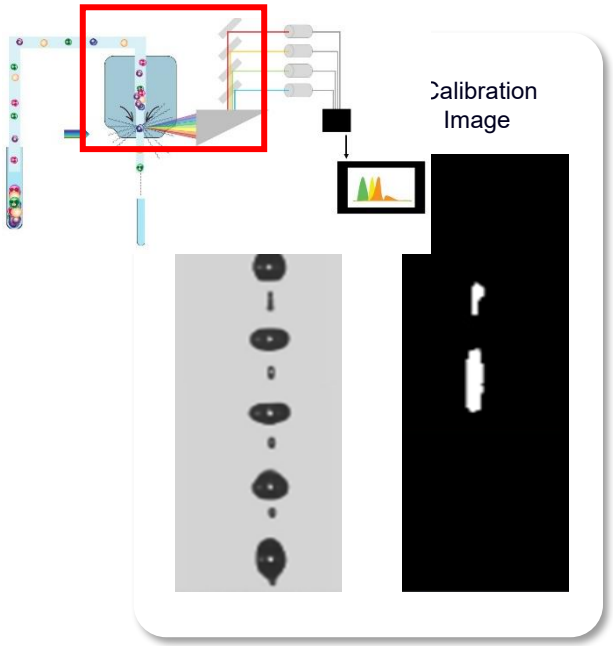


Fully automated system setup



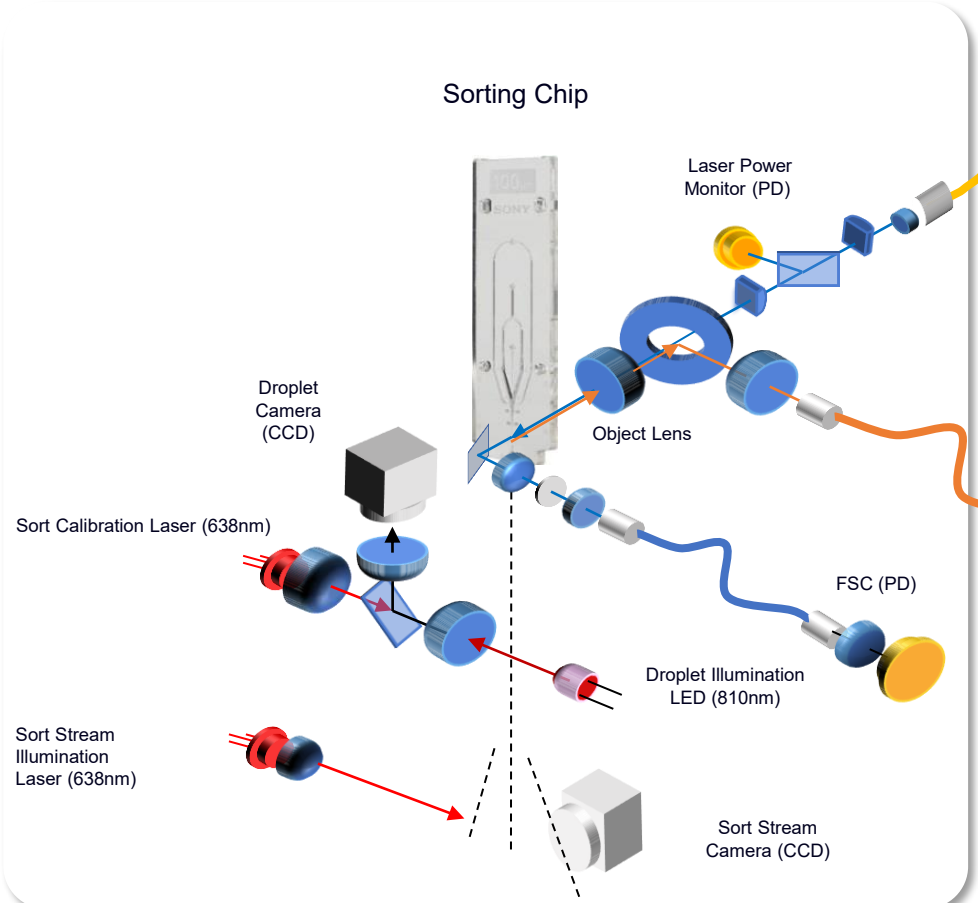
Fluorescence Detection
&
Compensation

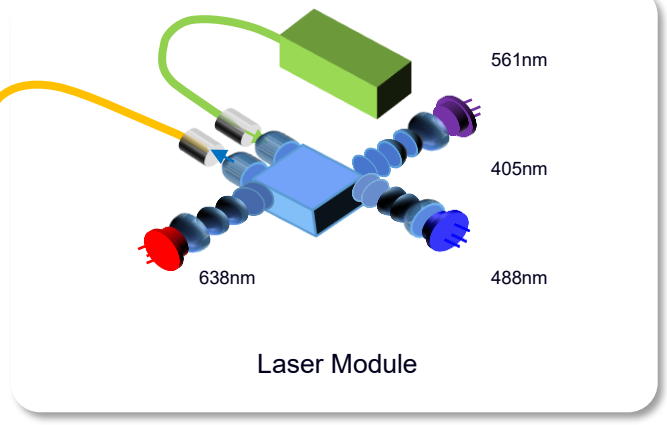
SH800 Optical Detection System



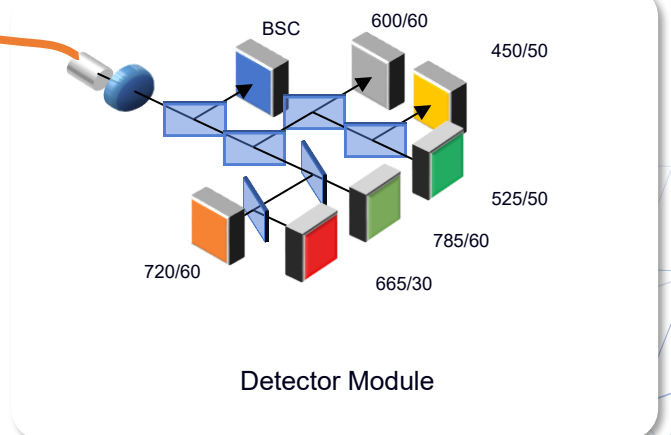


Sort Stream Image





Laser Module

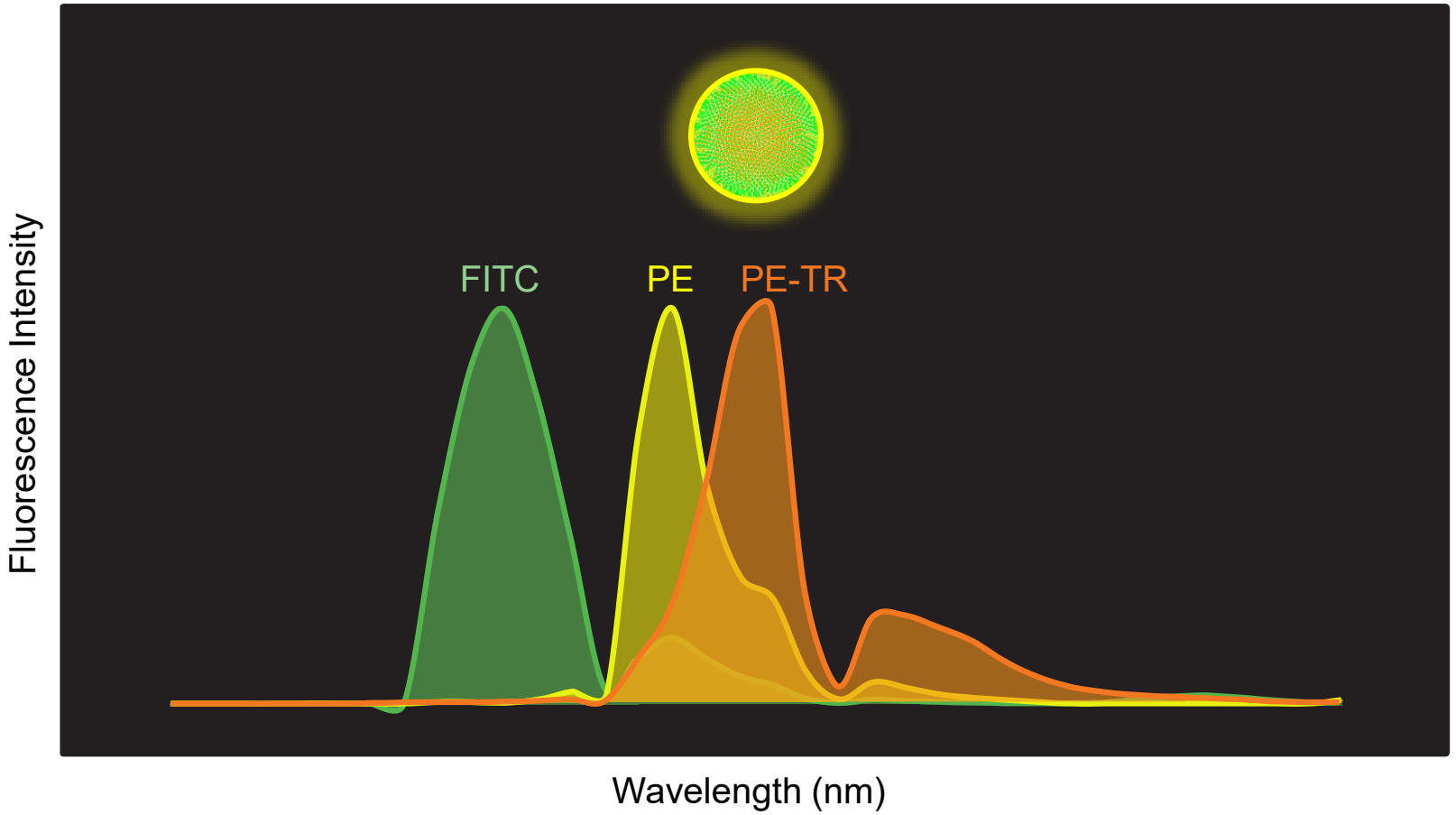


Detector Module

Fluorescence & Compensation

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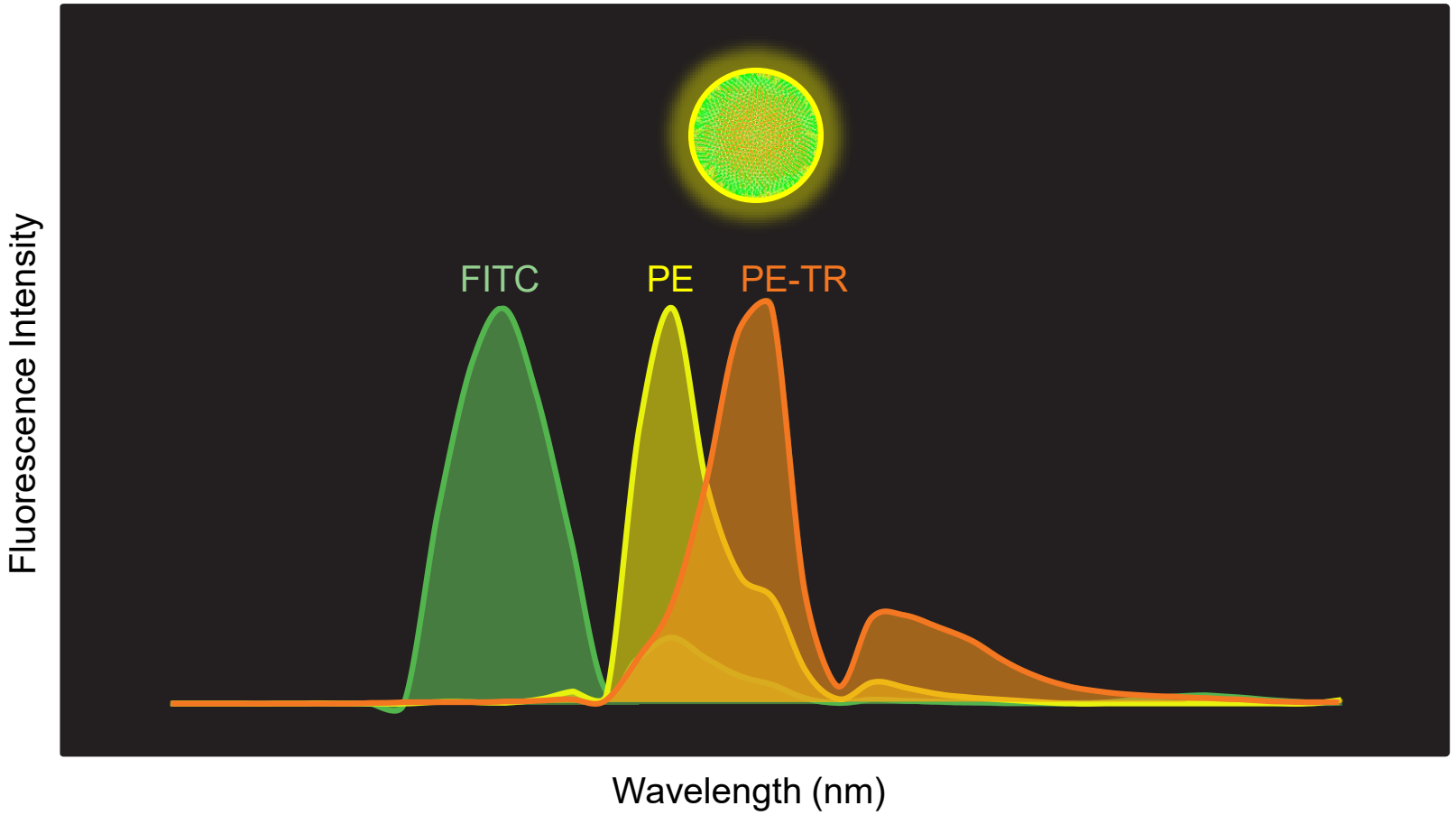
Consider the fluorescence from one cell stained with three fluorochromes.



Fluorescence & Compensation

SONY

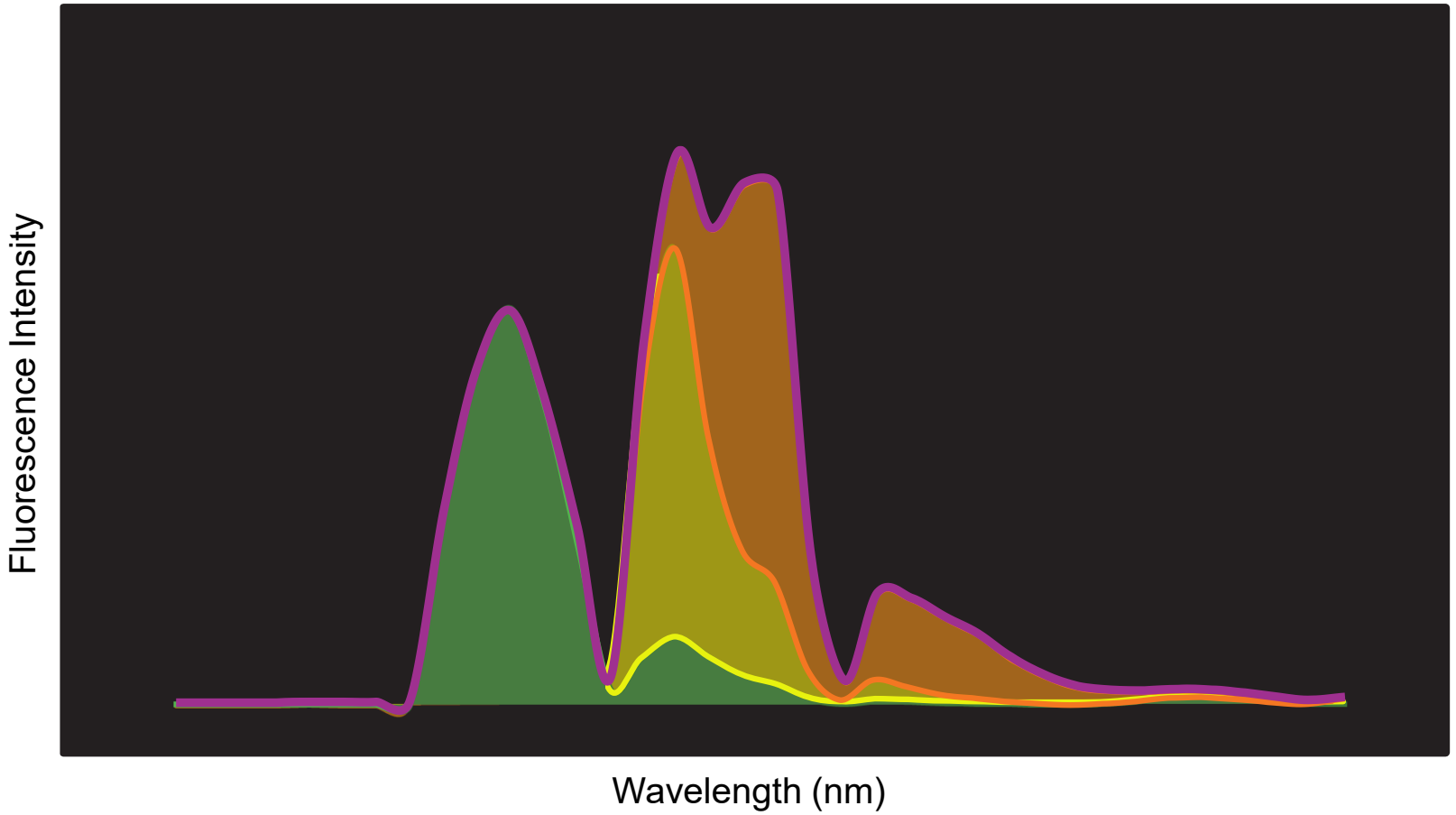
Measuring the intensity of each color is complicated by overlapping emissions.



Fluorescence & Compensation

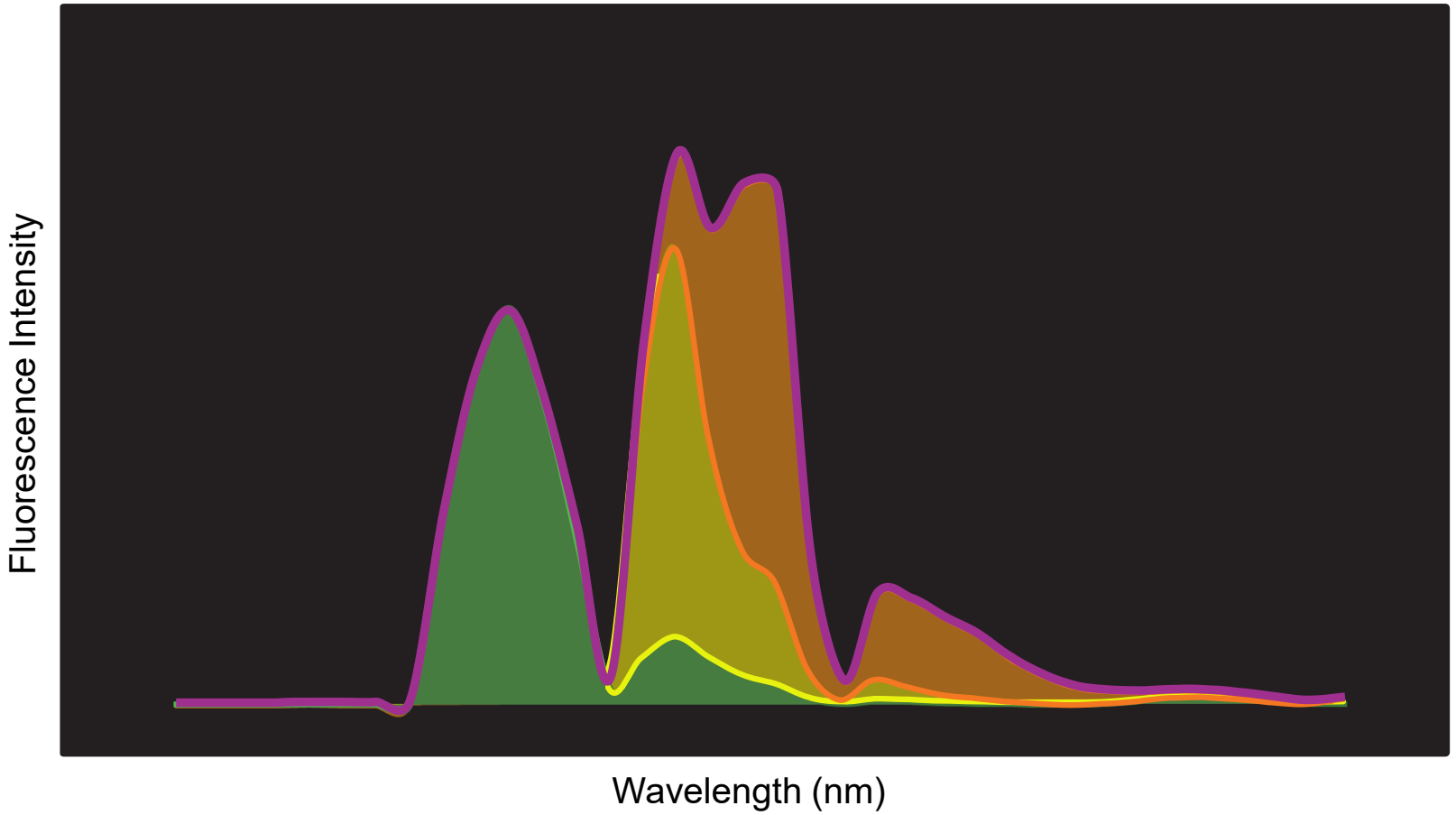
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In reality, the emissions sum together wherever they overlap.

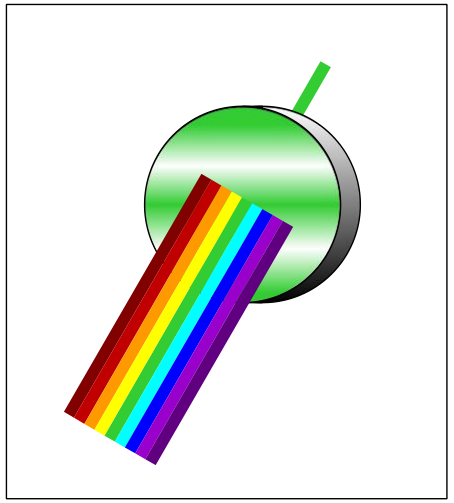


Fluorescence & Compensation

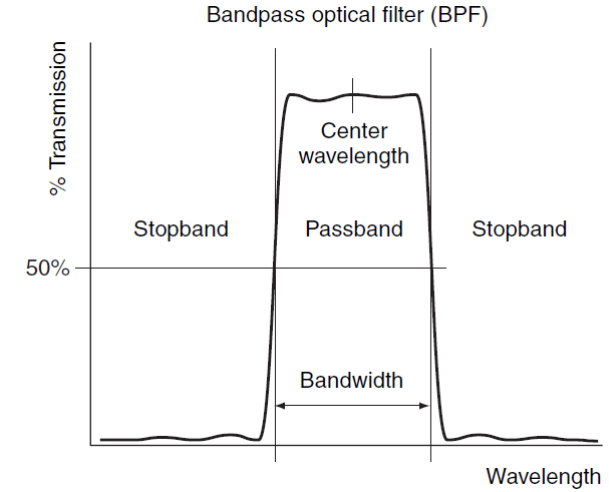
How do we measure the intensity of each individual colour?



Fluorescence & Compensation

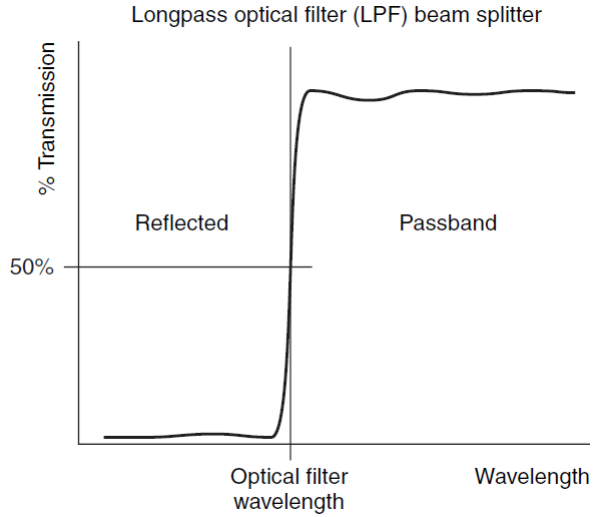


- **Band-pass filters**
- BP600/60
  - 600 nm centre
  - 60 nm bandwidth
  - ± 30 nm = 570– 630 nm

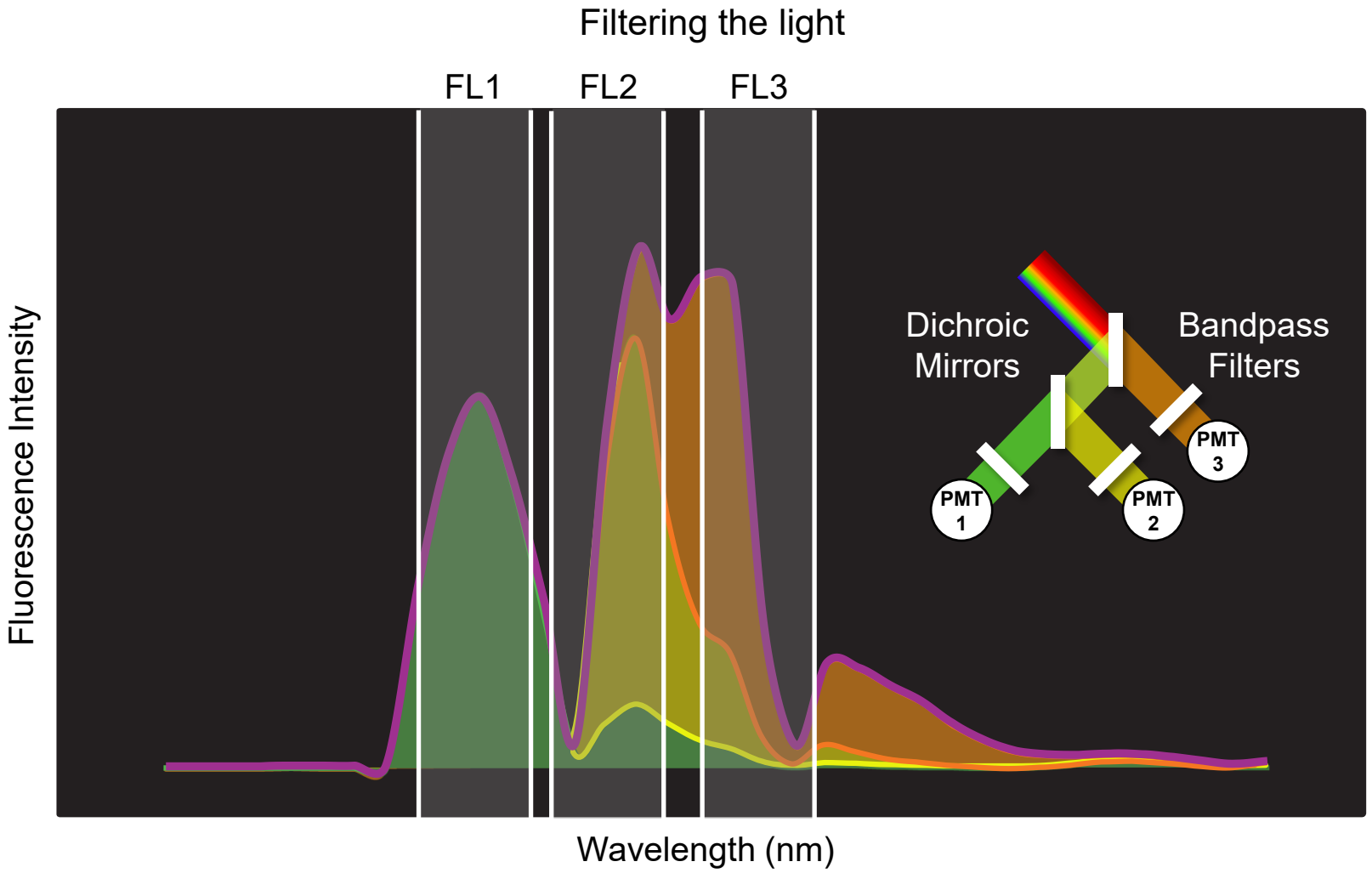




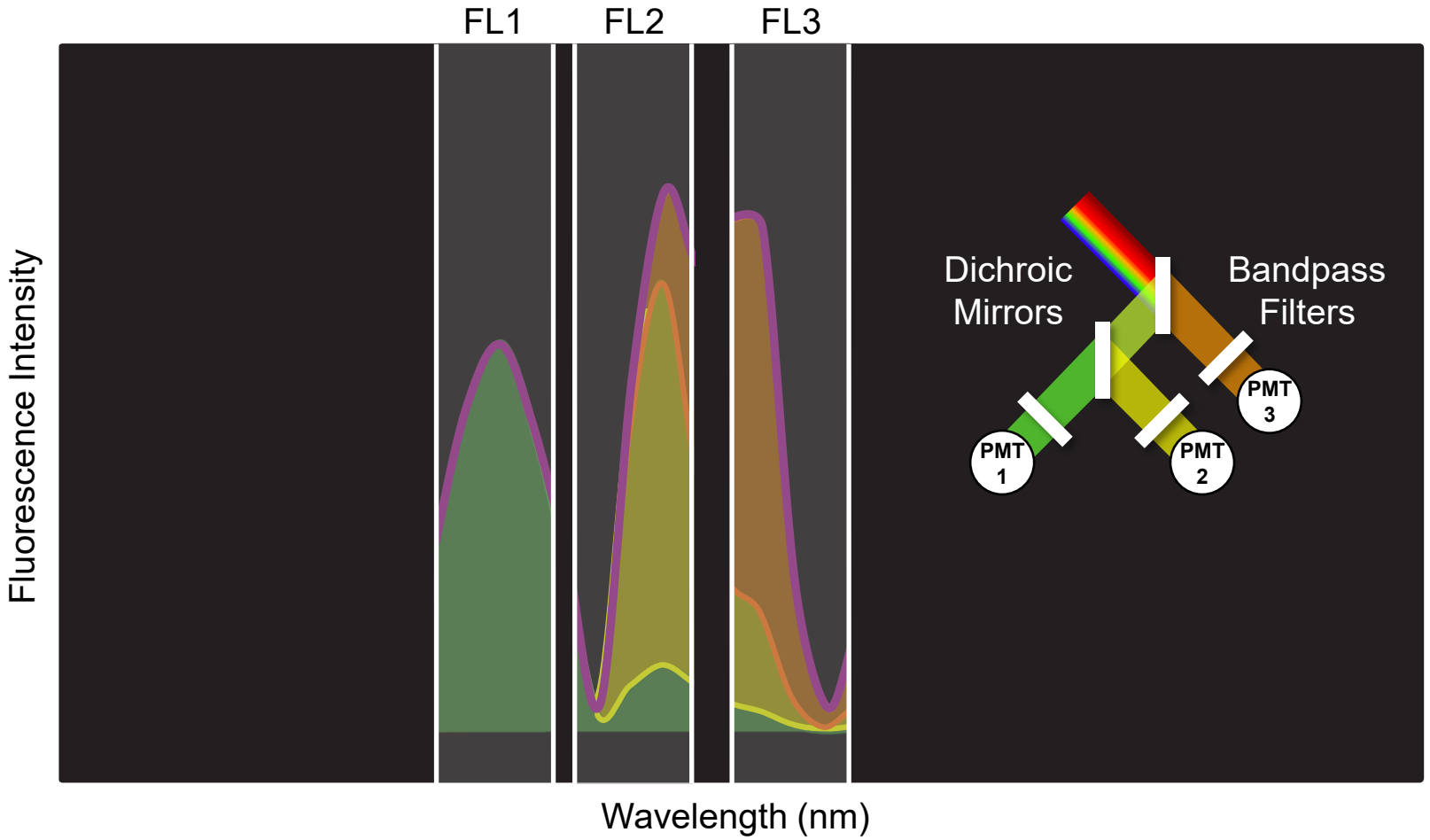
- **Long-pass filters (Dichroic Mirror)**
- DC450LP
  - 450 nm long pass



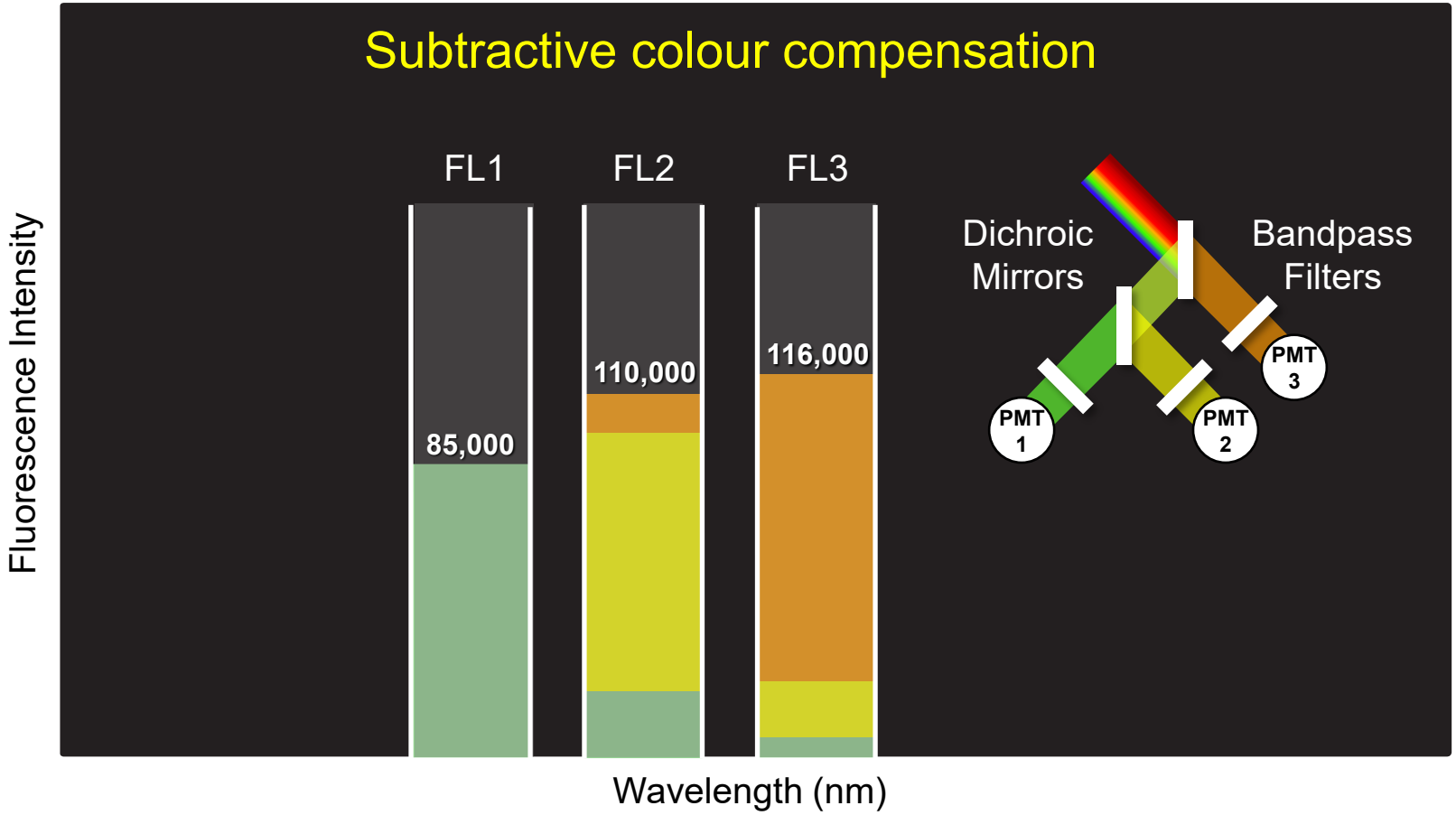
Fluorescence & Compensation



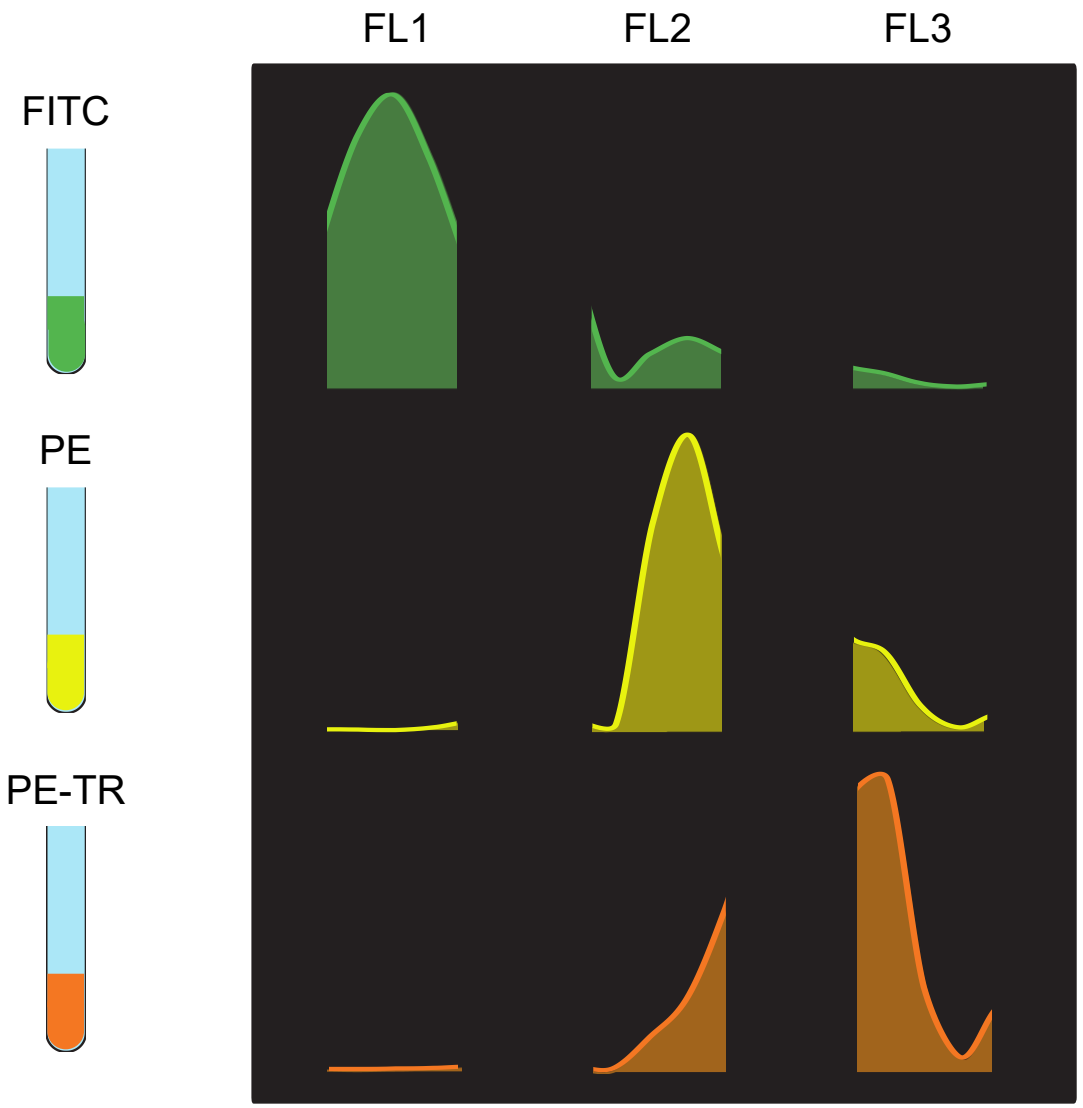
Fluorescence & Compensation



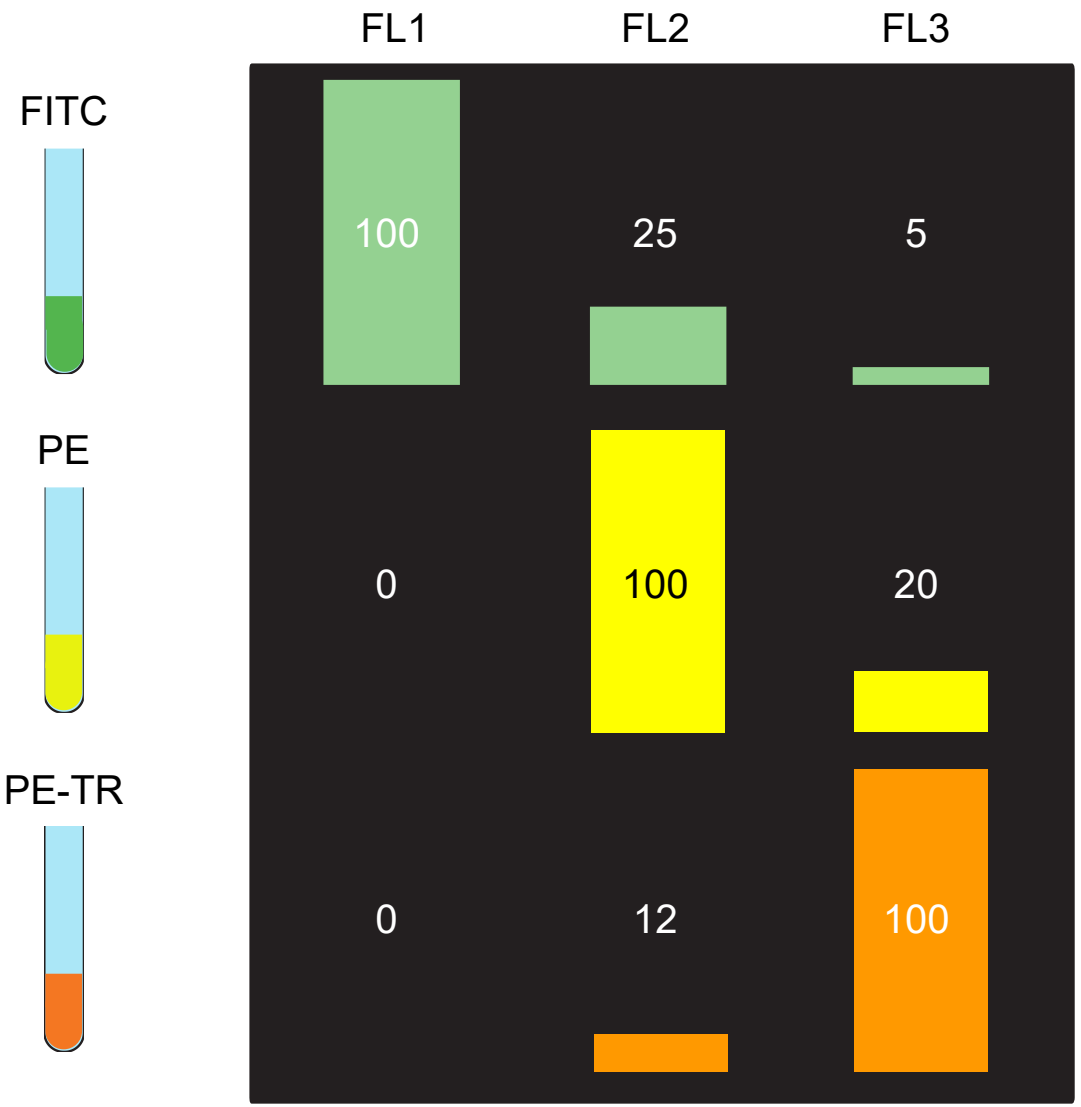
Fluorescence & Compensation



Fluorescence & Compensation



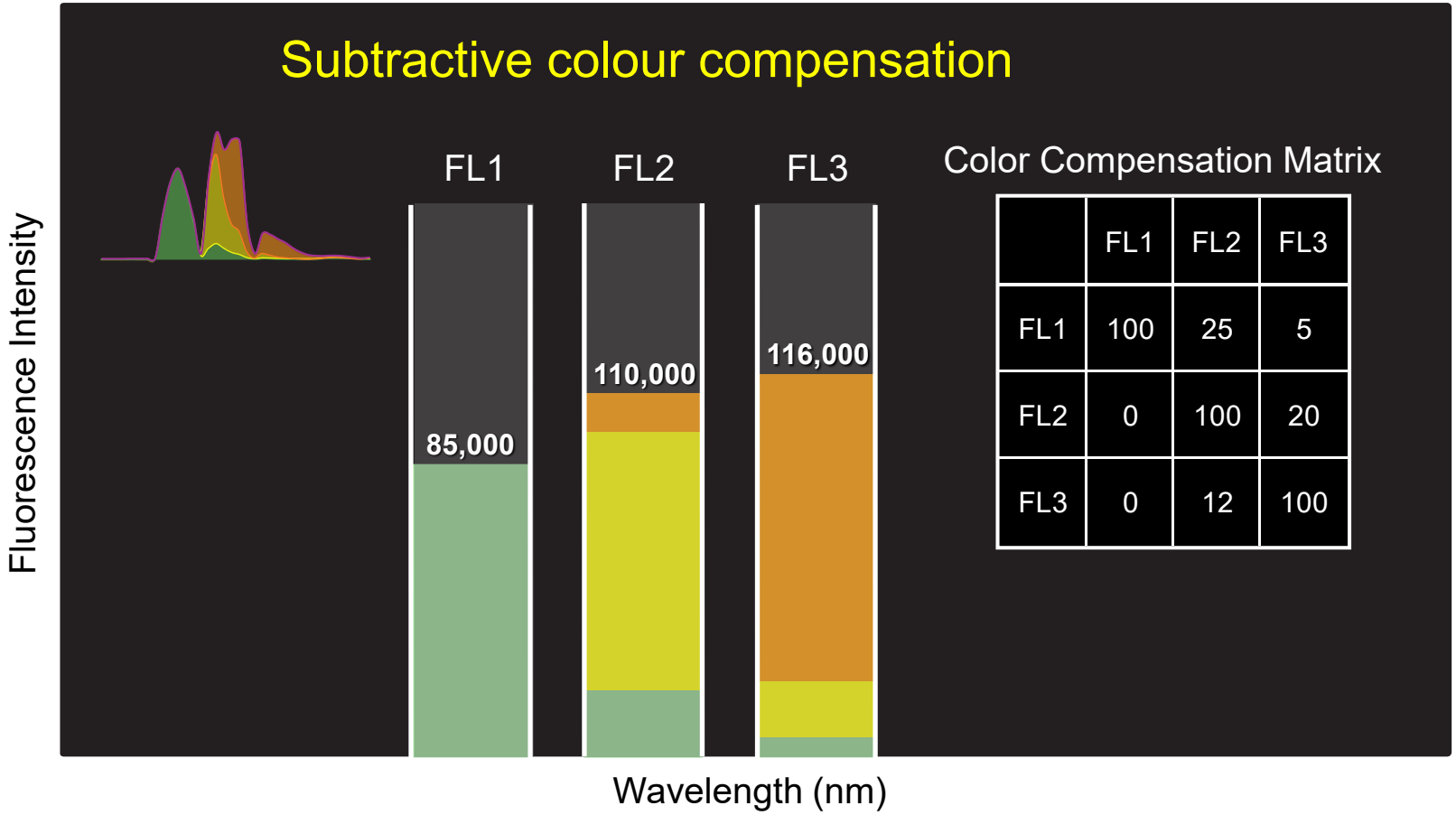
Fluorescence & Compensation



Color Compensation Matrix

	FL1	FL2	FL3
FL1	100	25	5
FL2	0	100	20
FL3	0	12	100

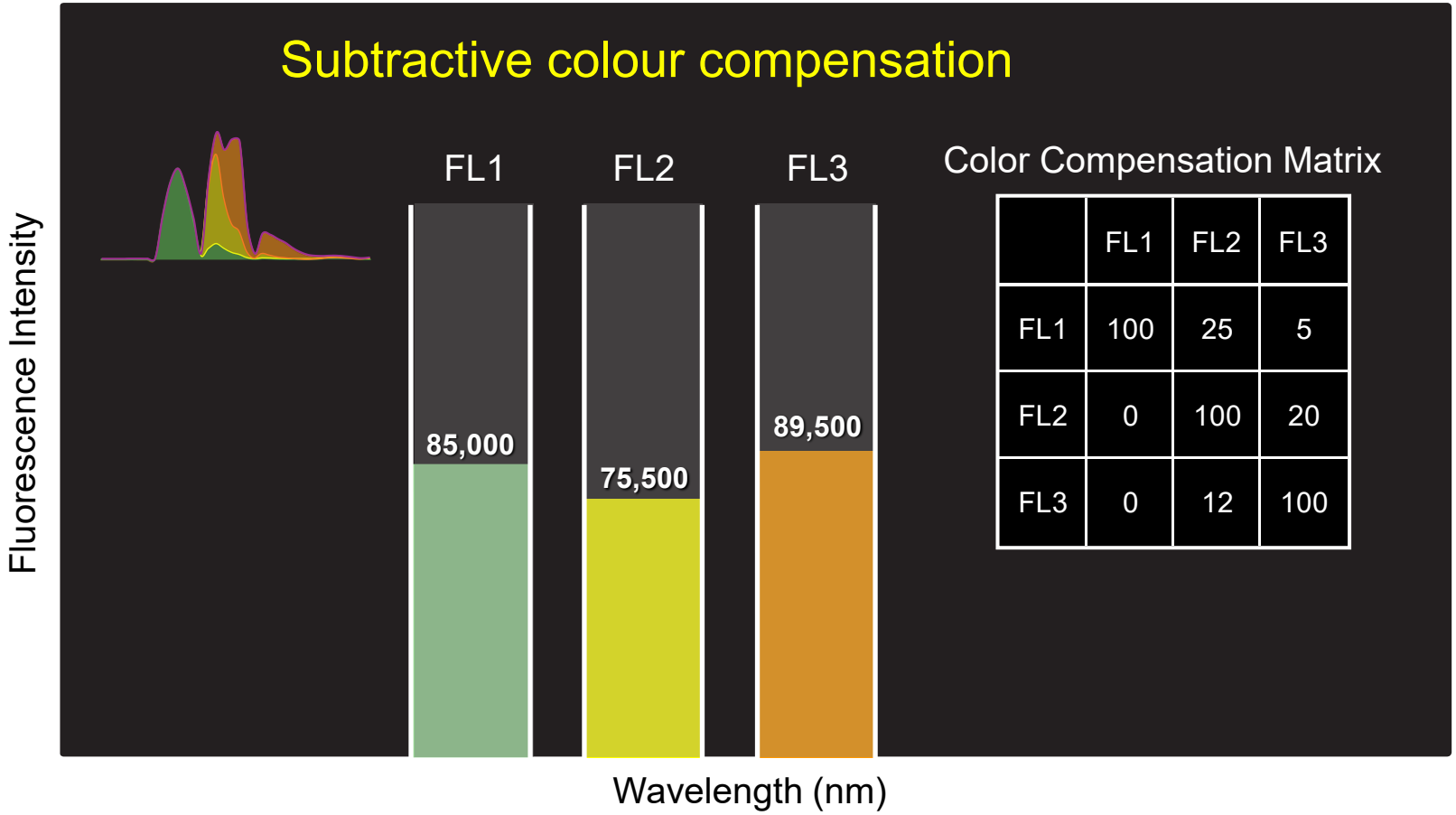
Fluorescence & Compensation



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Fluorescence & Compensation



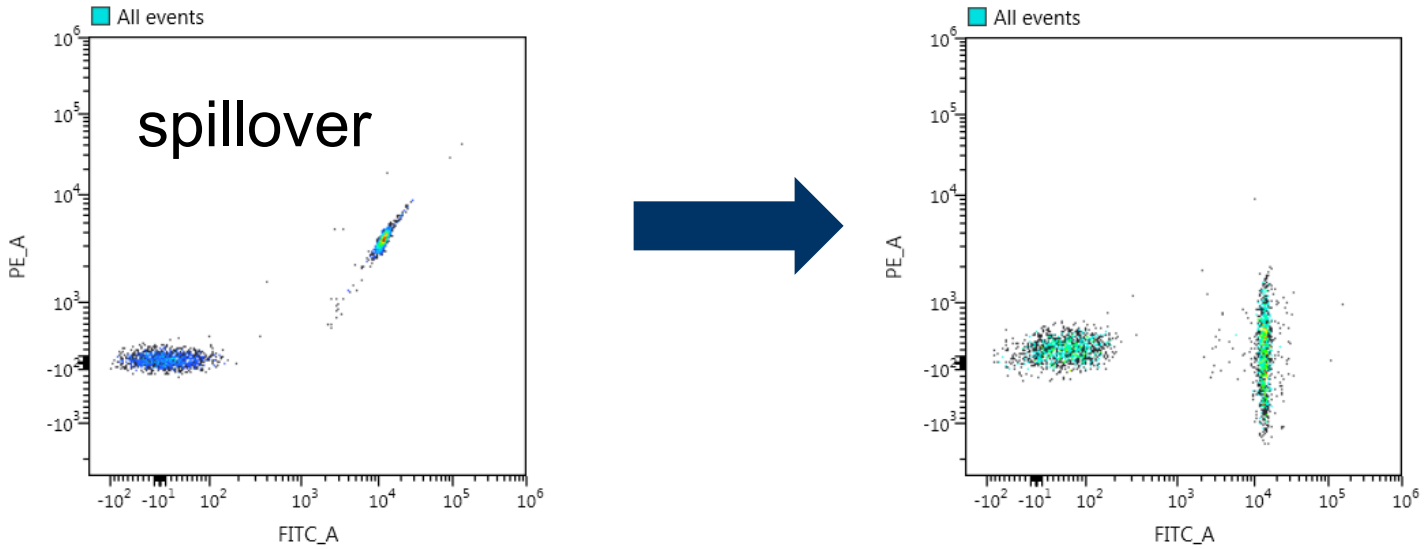
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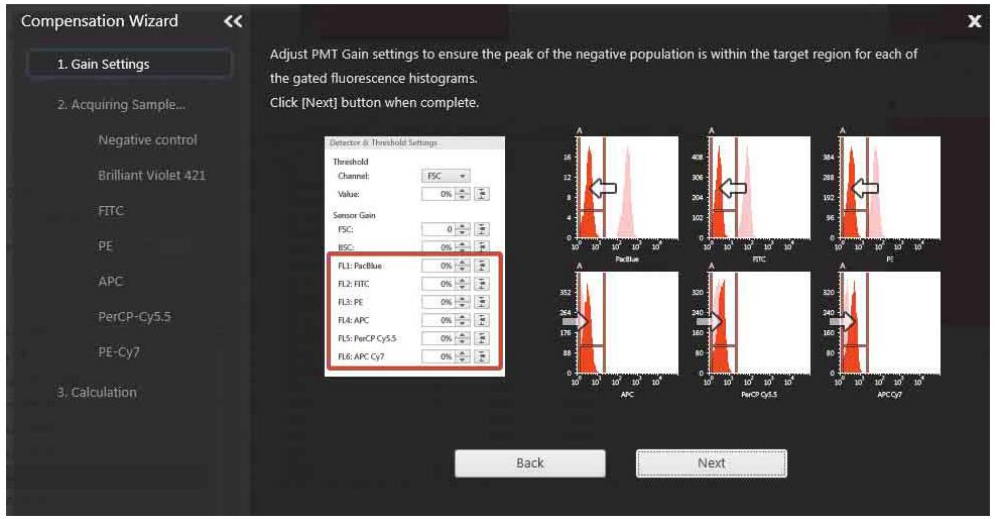
Fluorescence & Compensation

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Compensation = mathematical process used to determine a compensation matrix which effectively subtracts/corrects background due to spillover in all detectors.

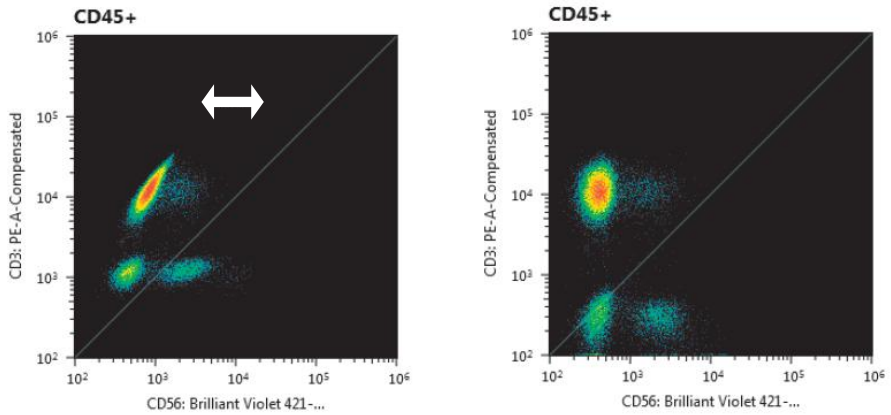


Compensation Wizard



A few steps through the automatic compensation procedure…

…but if needed, the fluorescence compensation can also be adjusted manually



SH800 Manual Compensation

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Compensation Settings

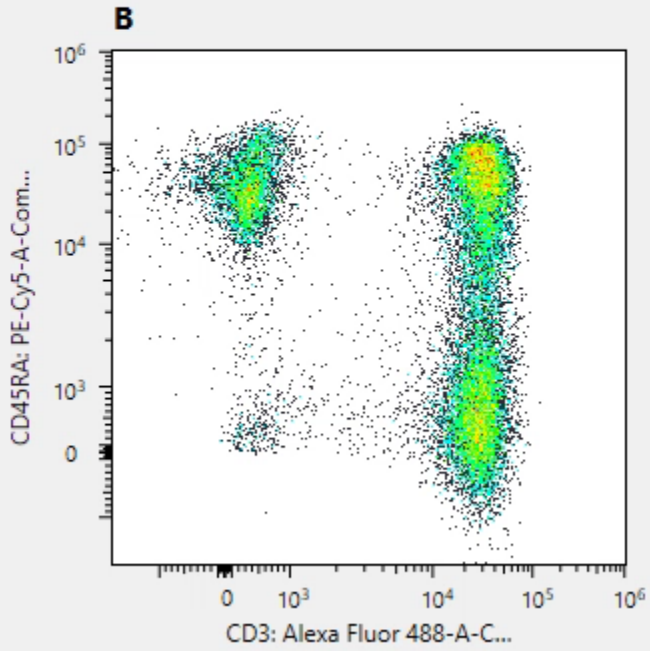
Spillover Matrix (%)

Fluorochrome \ Detector	HLADR: Bril...	AF 488	PE	APC	AF 700	PE-Cy7
HLADR: Bril...	100.00	4.27	0.30	0.03	-0.04	0.00
CD3: AF 488	0.06	100.00	15.39	1.12	0.37	0.13
CD16/56: PE	0.08	1.24	100.00	8.53	4.23	0.65
CD19: APC	0.74	0.18	0.75	100.00	38.96	9.08
CD8: AF 700	-0.06	-0.05	0.02	1.45	100.00	27.46
CD4: PE-Cy7	0.88	0.17	3.84	0.89	4.90	100.00

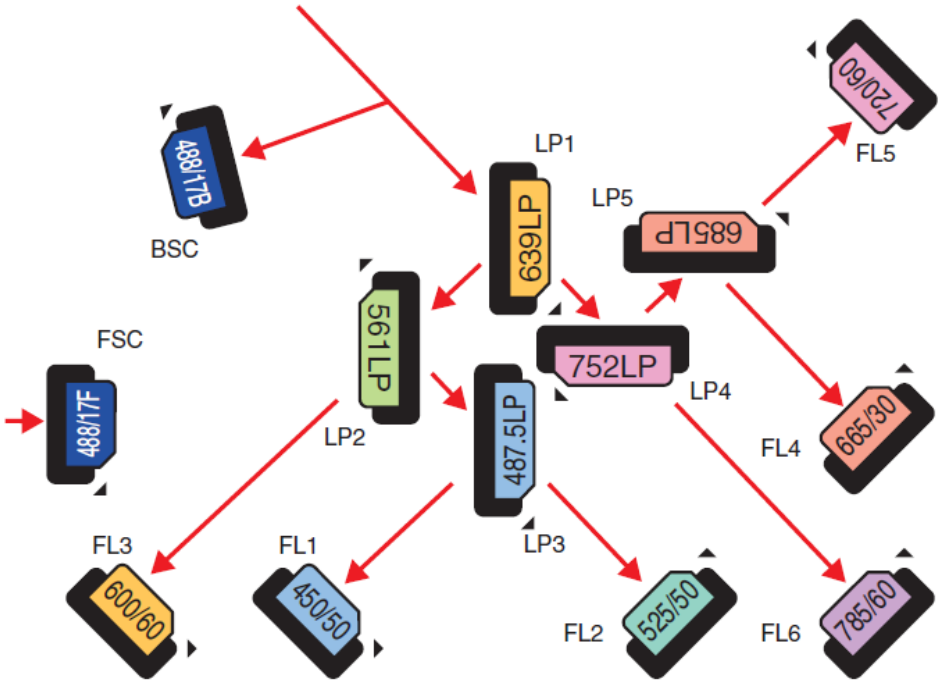
Negative Value

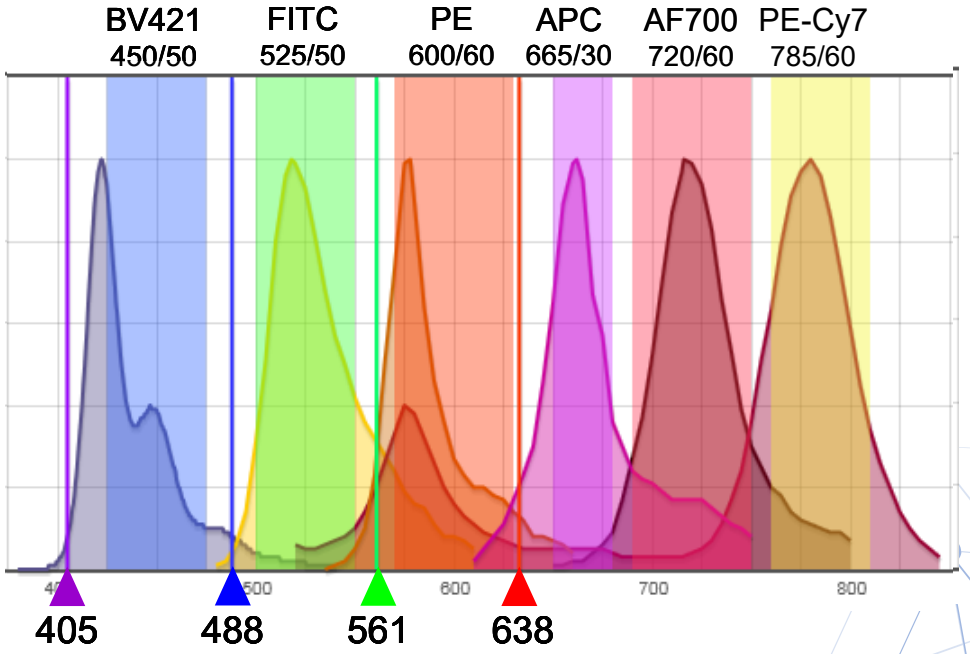
Save Load Reset

Close



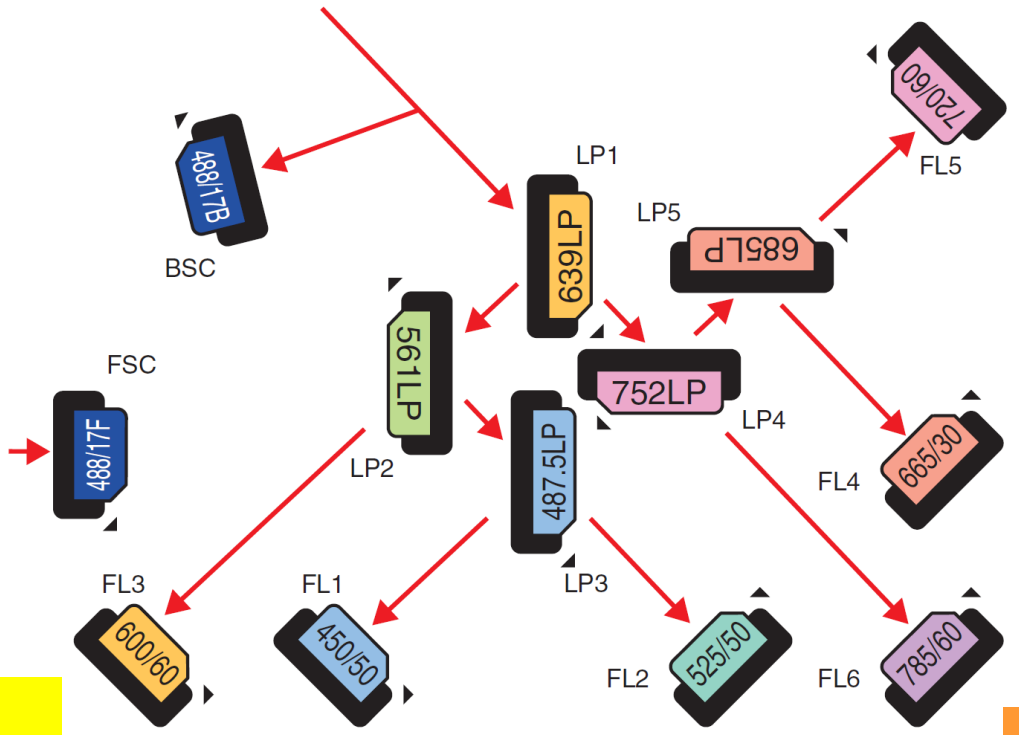
Standard Filter Configuration – 4 lasers





Standard Filter Configuration – 4 lasers

SONY



Alexa700
PerCP-Cy5.5
PE-Cy5.5
BV711

APC
AF647
PE-Cy5
PerCP
7AAD
mPlum
BV650

PE
mCherry
tdTomato
DsRed
mOrange
PE-TxRed
PI
BV605
BV570

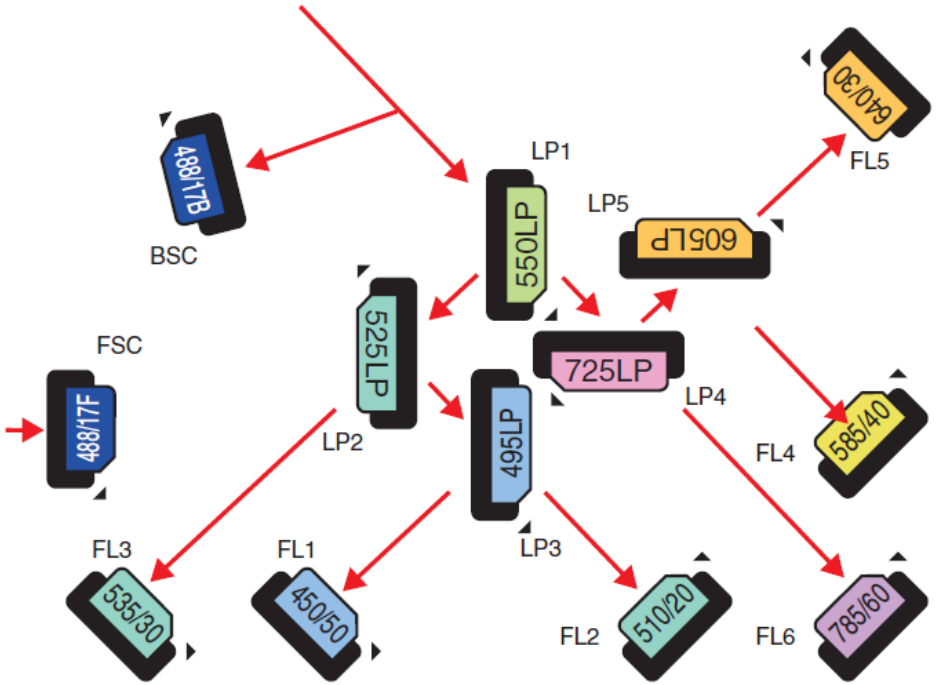
BV421
Pacific Blue
AF405
Hoechst
DAPI
CFP

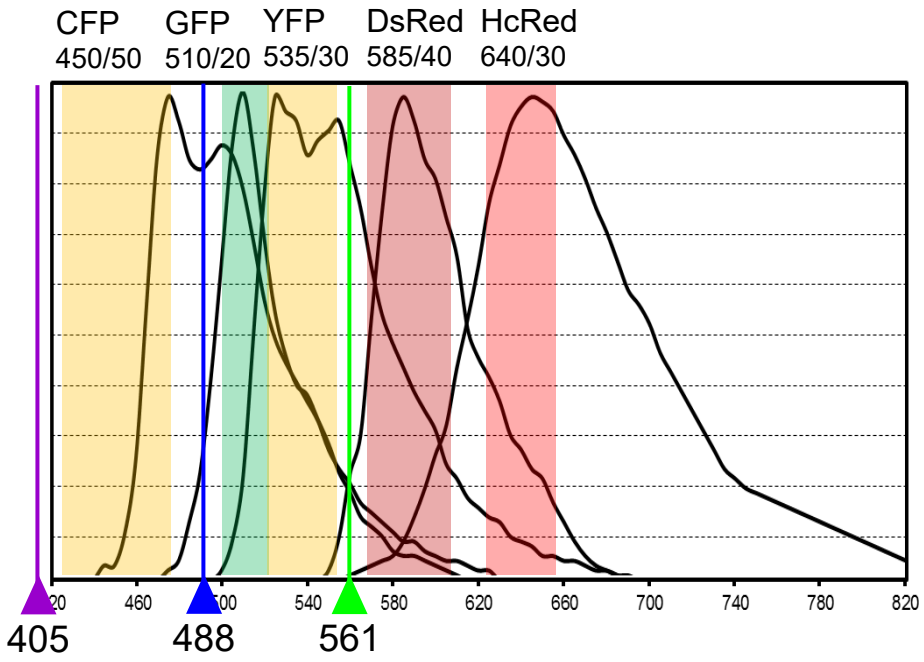
FITC
AF488
GFP*
YFP*
CFSC
BV510
mCitrine

PE-Cy7
APC-Cy7
APC-H7
BV785

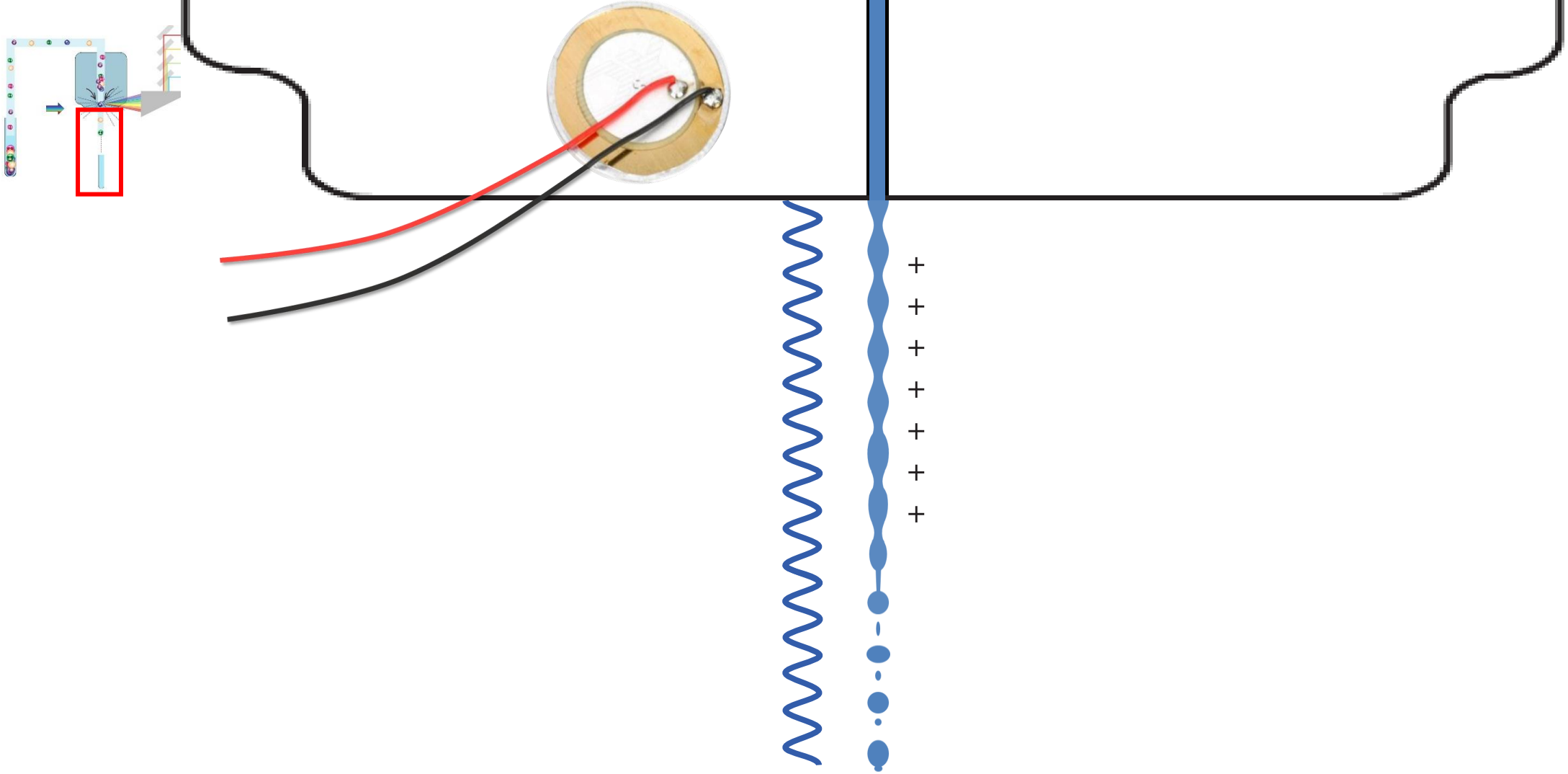
Fluorescent Protein Filter Configuration

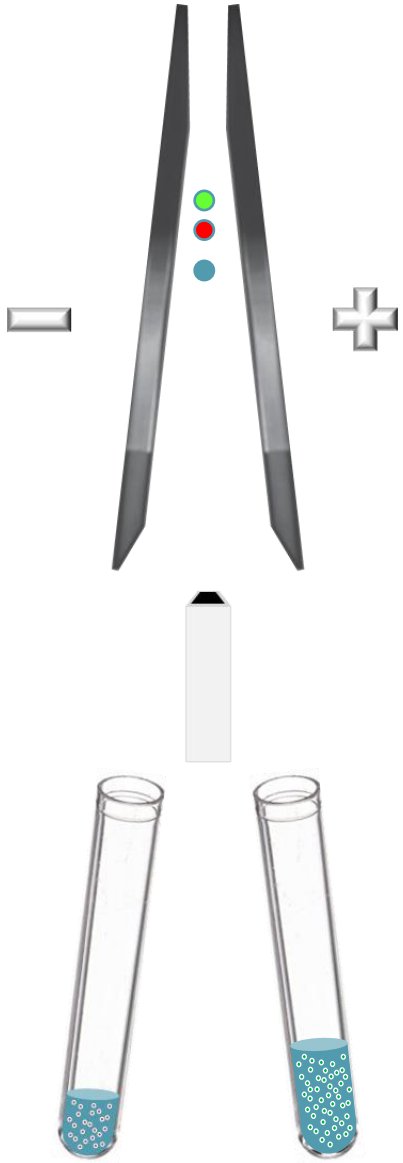
SONY

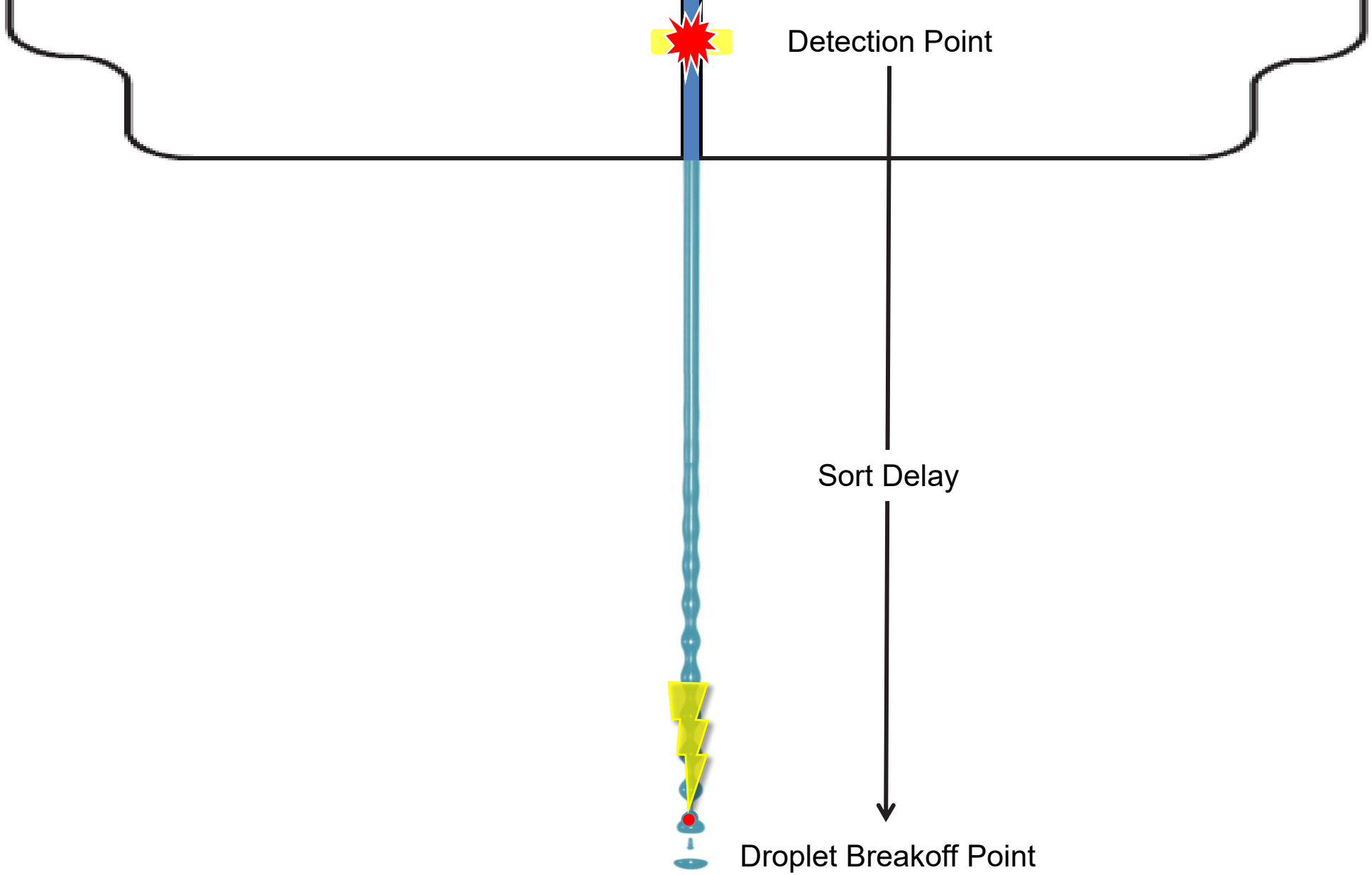


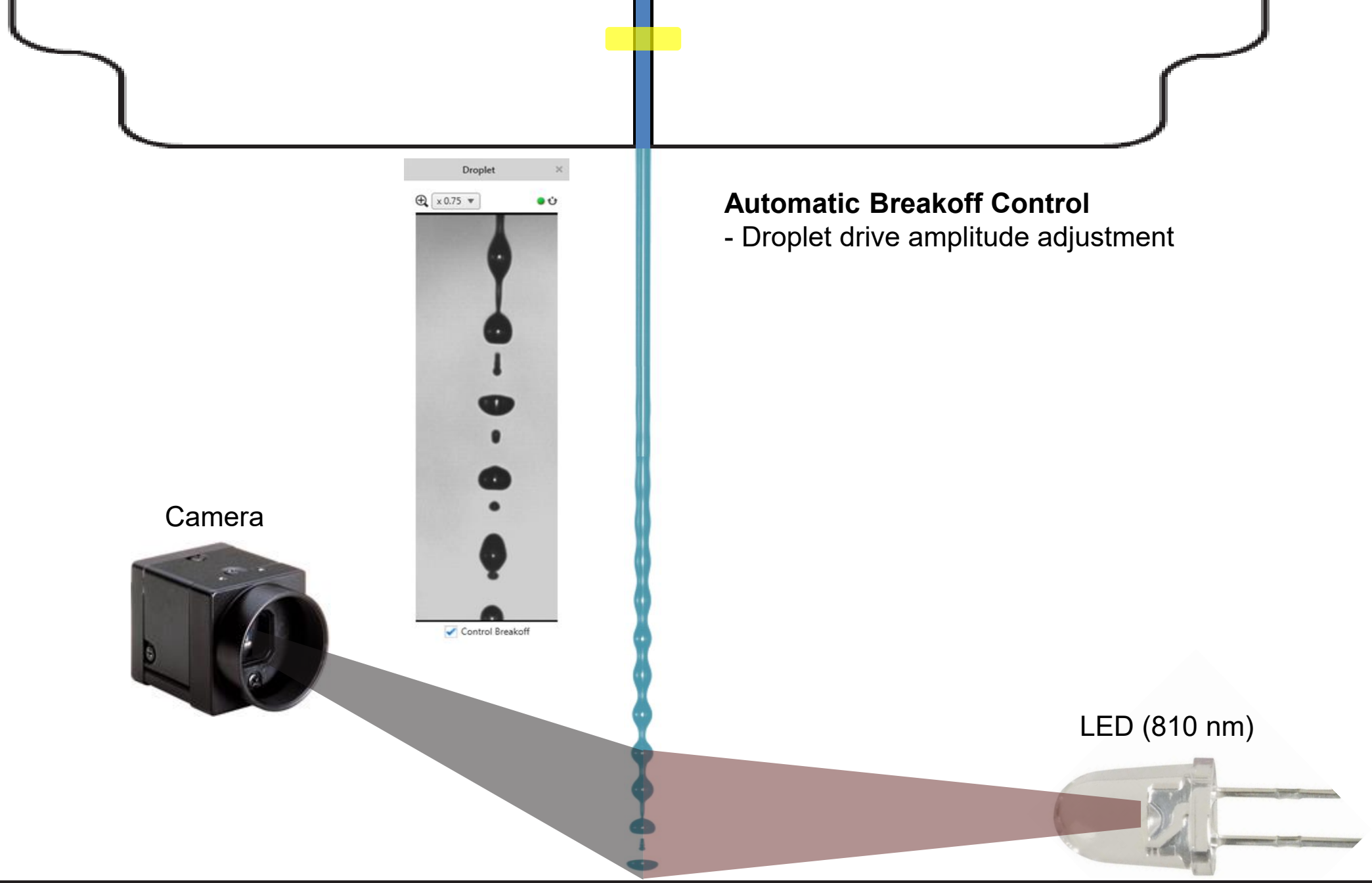


Basics of Sorting



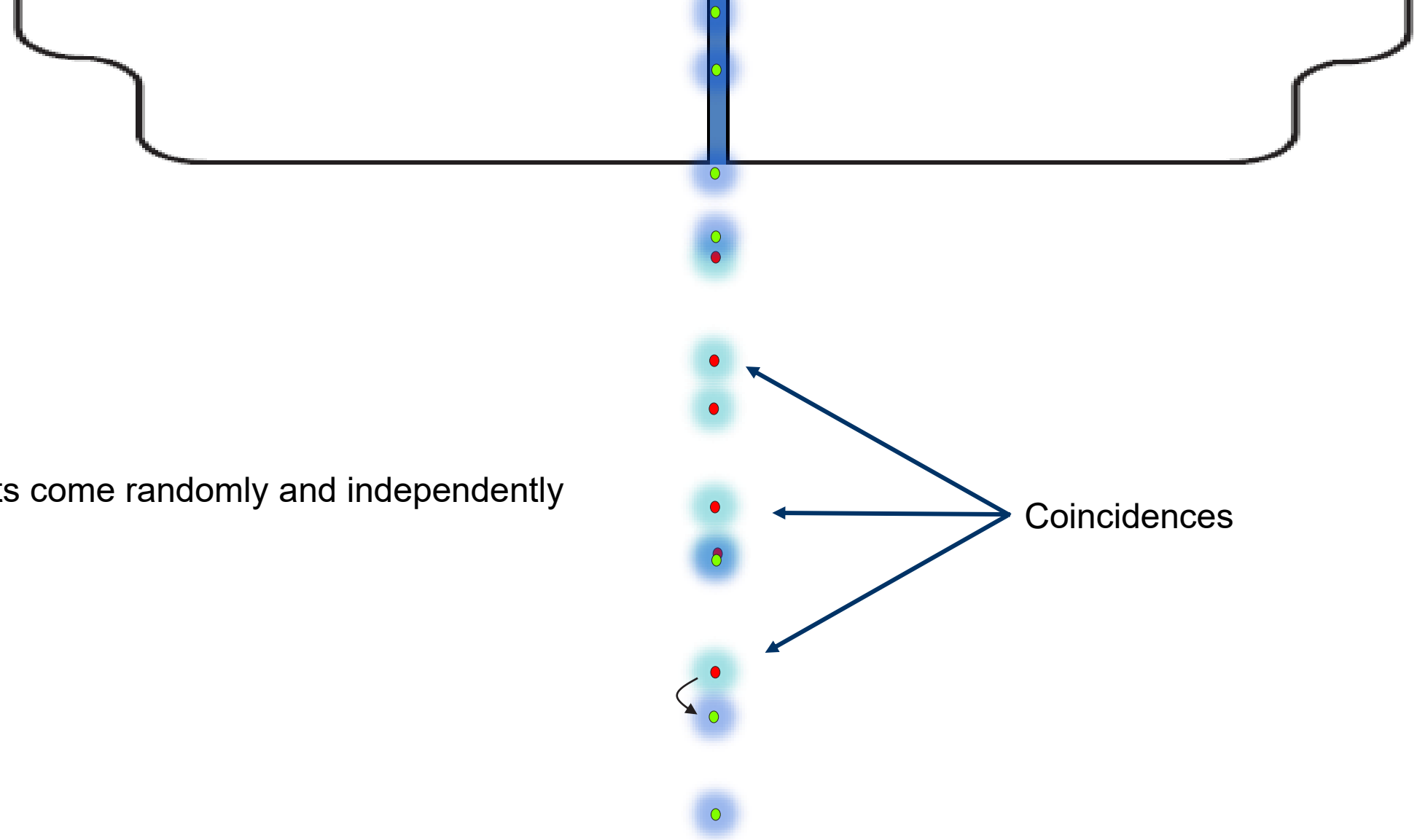



Detection Point
Sort Delay
Droplet Breakoff Point



Automatic Breakoff Control

- Droplet drive amplitude adjustment


Events come randomly and independently
Coincidences

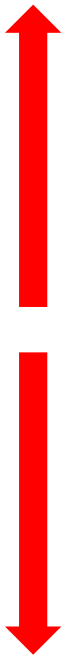
Sort Modes

SONY

What is the goal of my experiment?

1. Single Cell
2. Ultra Purity
3. Purity
4. Semi-Purity
5. Normal
6. Semi-Yield
7. Yield
8. Ultra-Yield

+ custom



Increasing Purity

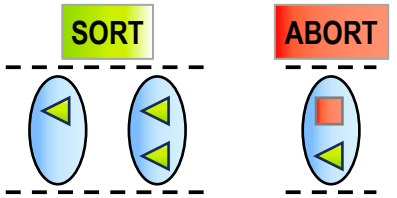
- *Purity* measures proportion of target cells collected as a percentage of all cells collected.
- Higher sort abort rate

Increasing Yield

- *Yield* measures proportion of target cells collected as a percentage of target cells in the sample.
- Lower purity

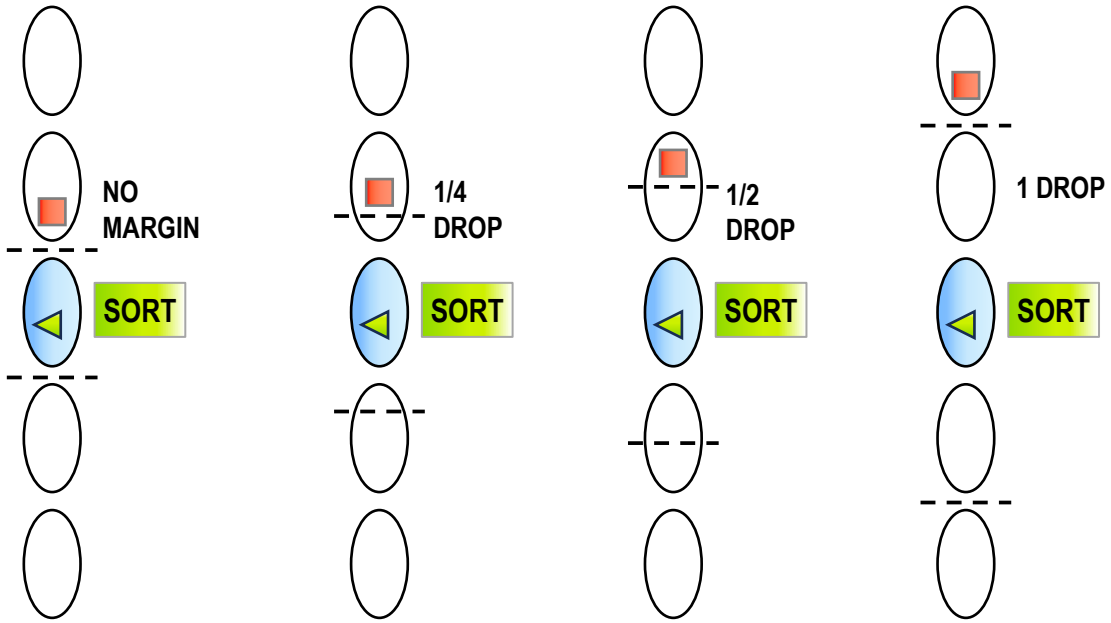
Sort Modes

PURITY MODES



- Purity is achieved at the expense of aborting the target◁ coincident with non-target■
- Suggested mode is Semi-Purity for best efficiency/purity trade-off
- For good efficiency, do not exceed sort speeds described in the nozzle selection guide

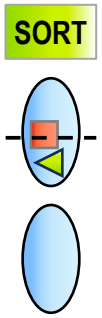
Normal	Semi-Purity	Purity	Ultra-Purity
~95% Purity ~90% Efficiency	~97% Purity ~80% Efficiency	~99% Purity ~70% Efficiency	~100% Purity ~60% Efficiency



Sort Modes

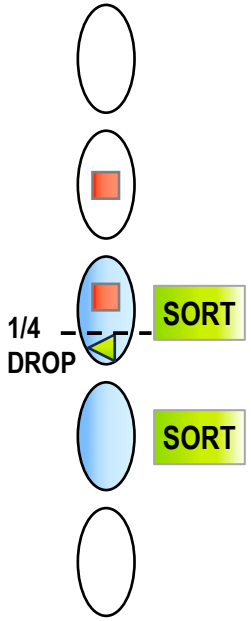
SONY

YIELD MODES

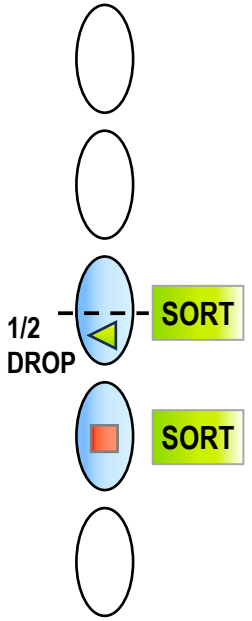


- Enrich for the target at the expense of purity
- Coincidence is not aborted
- Extra drops are sorted for maximum recovery
- Suggested mode is Semi-Yield
- You may exceed nozzle sort speed

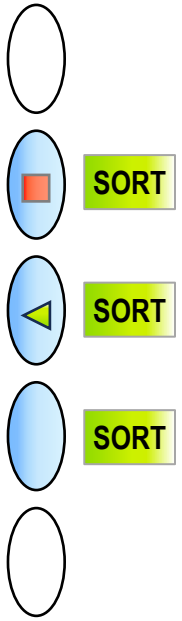
Semi-Yield
98% Efficiency
1/4 drop extension



Yield
99% Efficiency
1/2 drop extension

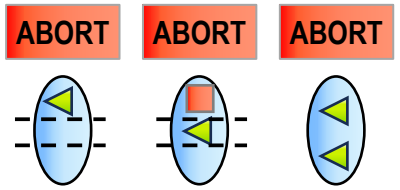


Ultra-Yield
100% Efficiency
3-drop sort



Sort Modes

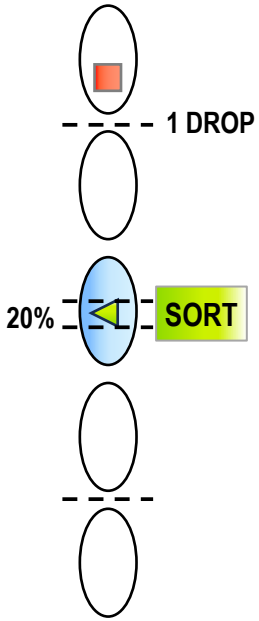
SINGLE CELL MODES



- Used for single-cell plate sorts
- Sort only 1 (not 2) targets, at 100% purity
- Centering Mask ensures target is in the sorting drop
- Expanding the Centering Mask increases efficiency
- 130 µm nozzle always sorts 3 drops in any single-cell mode

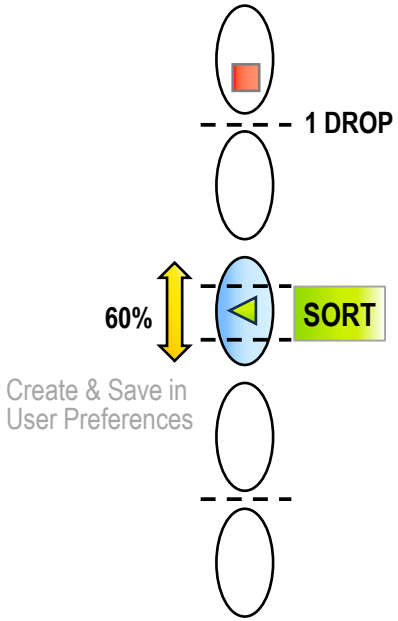
Single Cell

20% Centering Mask



Single Cell Custom

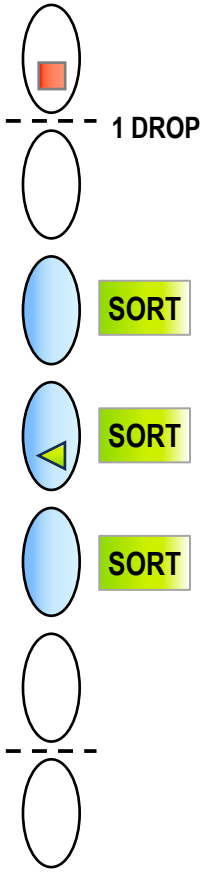
Customizable Centering Mask (e.g. 60%)



Create & Save in User Preferences

Single Cell 3-Drop

No Centering Mask 3-drop sort

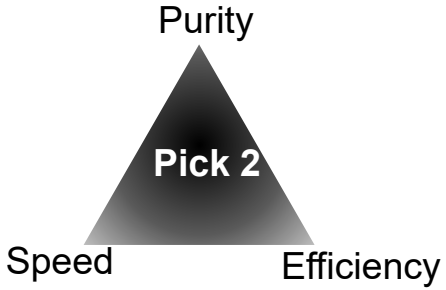


100 µm chip different side stream angle

General Considerations

SONY

Maximum event rate: 1/4 – 1/5 droplet frequency



Nozzle size	70 µm	100 µm	130 µm	
Mode		Standard	Targeted	
Drop frequency (Empty drops/second)	~50K	~30K	~22K	~12K
Max suggested* event rate (Total events/second)	12K	6K	<4K	2.5K
	*When sorting for purity, do not exceed the indicated event rate.			
Drop volume	1.5 nL	3 nL	4 nL	7.9 nL
Sorted events/1 mL (of sorted volume)	~700K	~333K	~250K	~40-125K

Sample pressure/Sample flow/Event rate

SONY

100 µm sorting chip

[Sample Pressure]		1	2	3	4	5	6	7	8	9	10
Flow rate (µl/min)		6	11	16	21	27	37	47	58	68	89
Event rate (eps)	Sample concentration (1e6/ml)	60	160	260	350	450	650	840	1,000	1,200	1,600
	Sample concentration (1e7/ml)	600	1,300	2,100	2,800	3,600	5,100	6,600	8,100	9,600	12,600

130 µm sorting chip

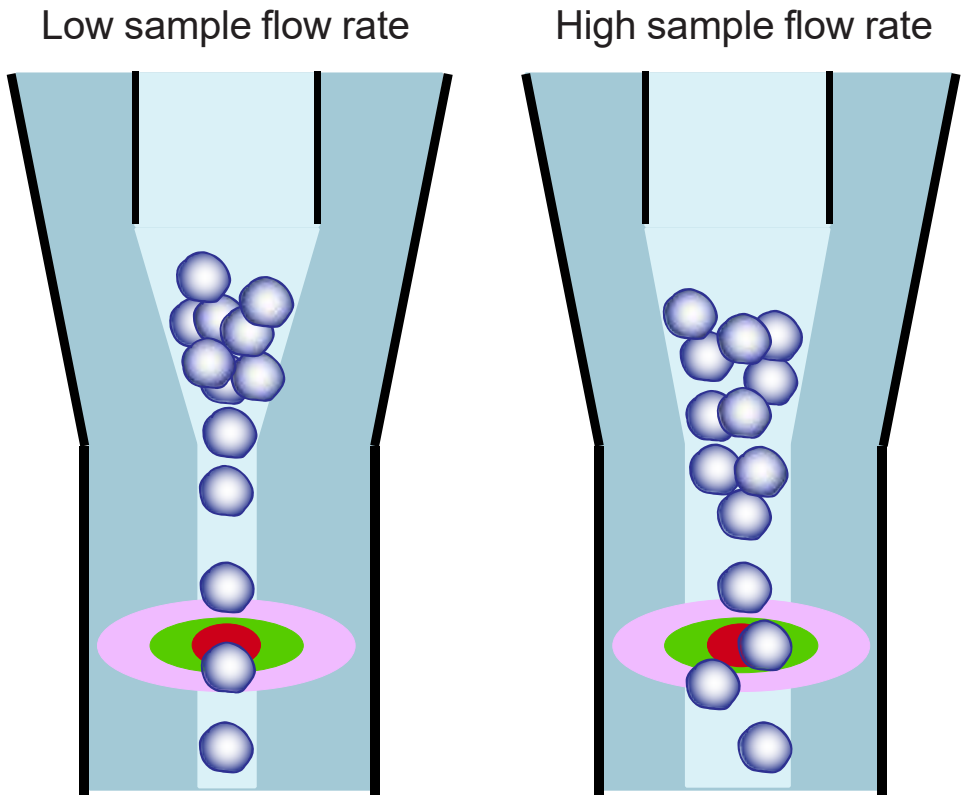
[Sample Pressure]		1	2	3	4	5	6	7	8	9	10
Flow rate (µl/min)		1	3	7	10	16	22	28	34	40	50
Event rate (eps)	Sample concentration (1e6/ml)	10	30	100	150	250	400	500	600	700	900
	Sample concentration (1e7/ml)	100	600	1,200	2,000	2,800	3,600	4,500	5,400	6,500	8,000

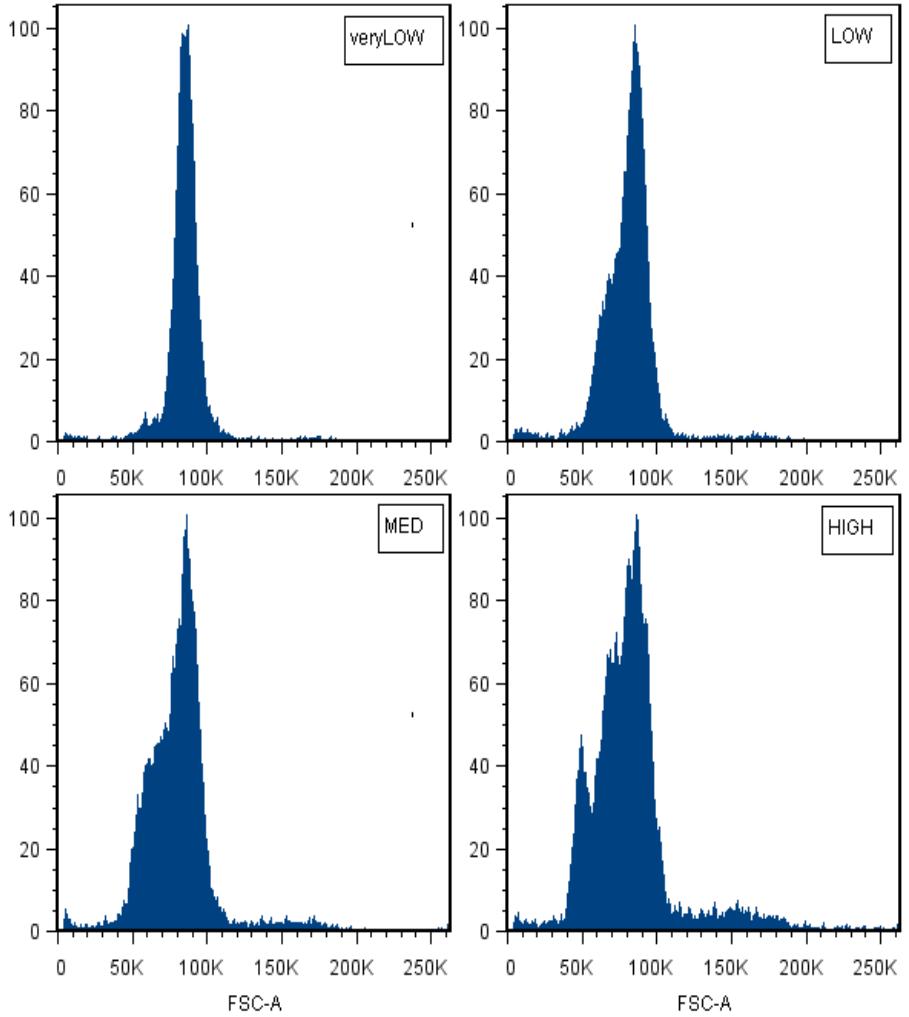
70 µm sorting chip

[Sample Pressure]		1	2	3	4	5	6	7	8	9	10
Flow rate (µl/min)		8	18	28	42	62	80	100	120	140	168
Event rate (eps)	Sample concentration (1e7/ml)	1000	2200	3500	5300	7700	10000	12500	15000	17500	21000

The values above are reference values. Values may vary depending on the environment.

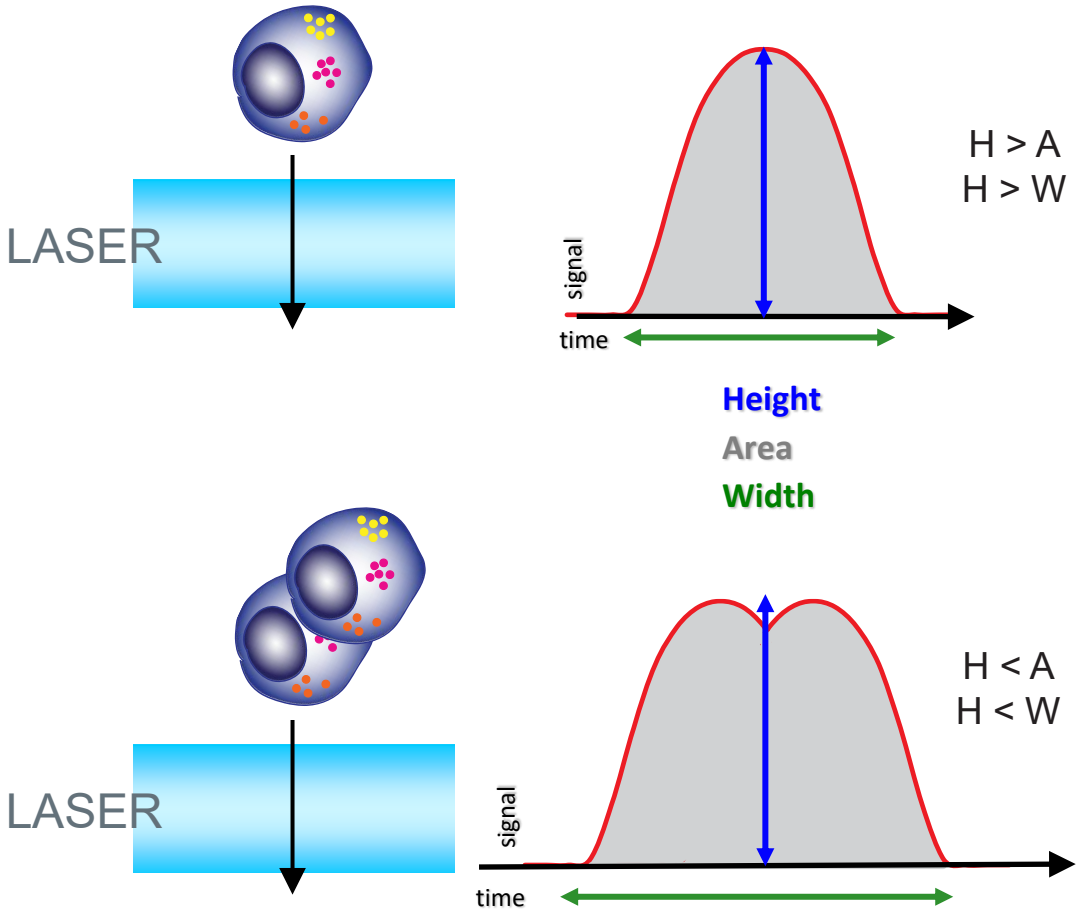
Sample Pressure

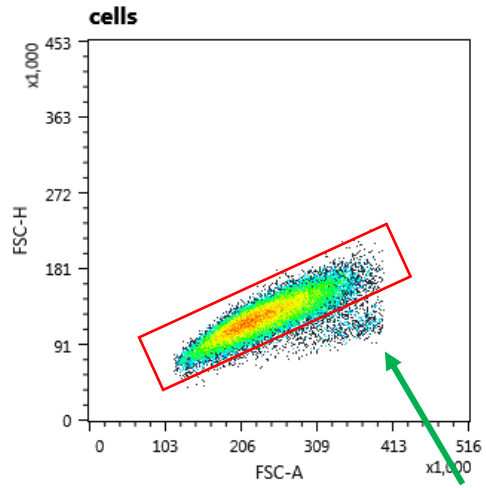




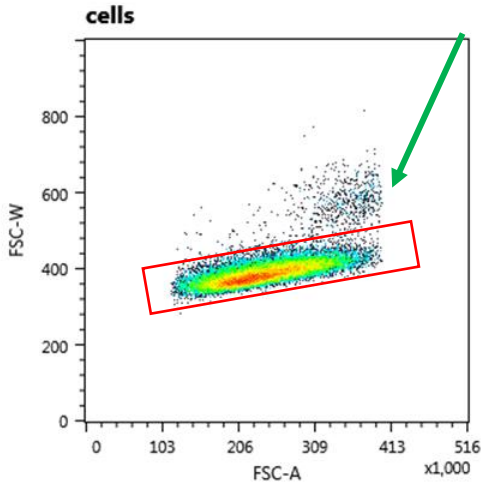
Other Considerations

Gating Strategy: doublets



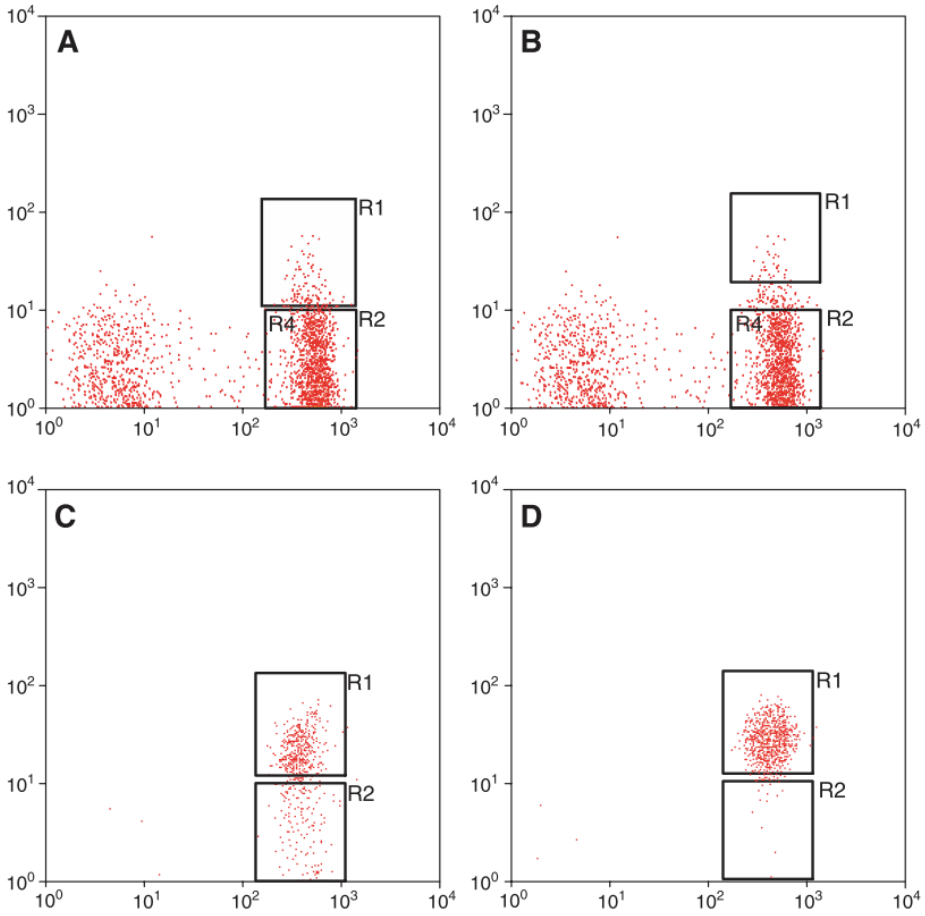


Doublets



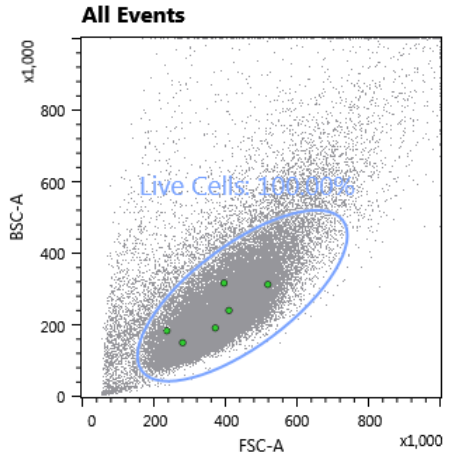
→ influences purity

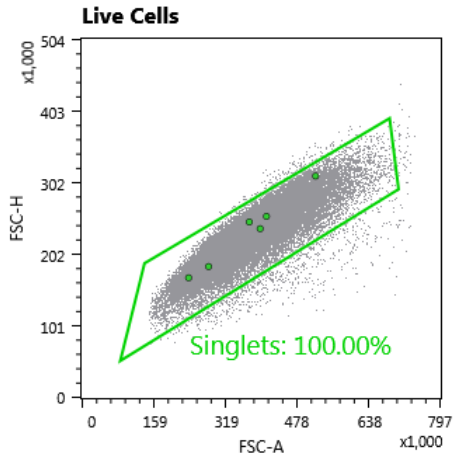
Gating Strategy: dim/negative populations

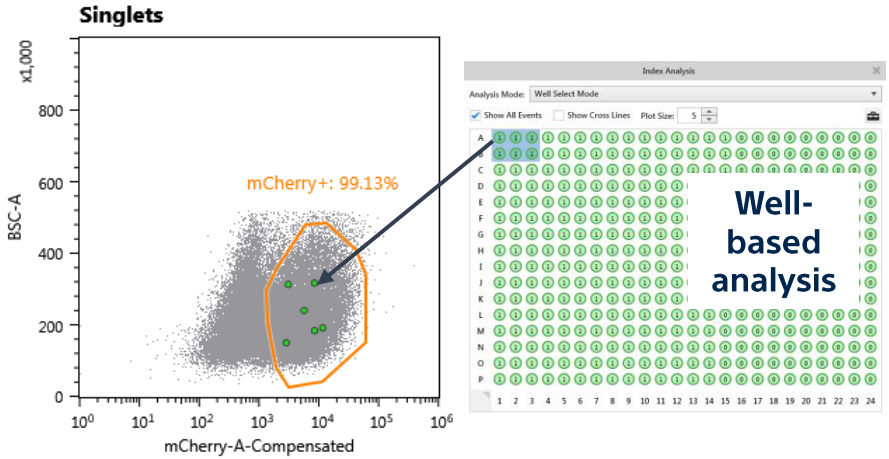


https://www.ncbi.nlm.nih.gov/pubmed/20069524

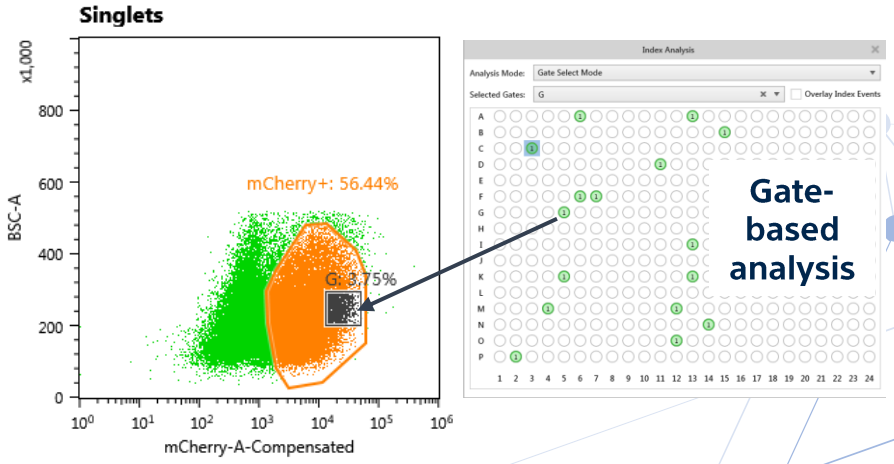
Index Sorting







- Records the X and Y coordinates of sorted cells
- Enables tracking of individual sorted cells to scatter/fluorescence
- Exporting data as .csv files



Sample Preparation for Sorting

SONY

Optimise to achieve high viability, low clumping and little debris!!

Prepare concentrated sample, e.g. 1x10^7 cells/ml

- Can be diluted later during the sort
- Minimise centrifugation

Clumping

- Filter your samples (30-50 µm mesh)
- Prevention: EDTA, DNase
- Keep on ice + use onboard agitation and temperature control

Exclude dead cells (PI, DAPI,..)

Sorting buffer: PBS/HBSS

2% FBS

2 mM EDTA

Sample collection

- Avoid long sorts
- Collection medium (culture vs PCR, dilution, mixing)

Large Particle Upgrade

Introduction - Large Particle Sorting

Traditional cell sorters have challenges with sorting large particles leading to reduced efficiency, recovery, and yield. These challenges are:

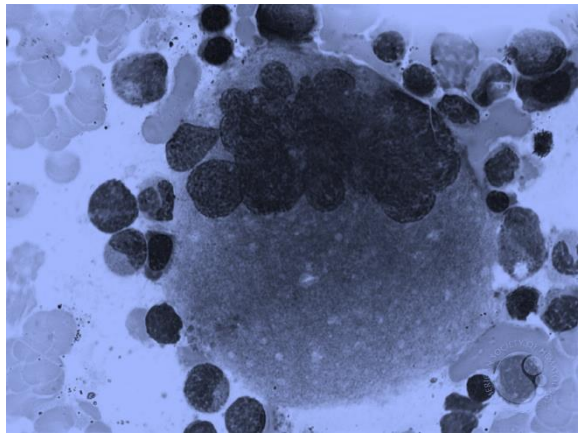
- ⚠️ Unstable droplet formation
- ⚠️ Nozzle clogging
- ⚠️ Inaccurate event detection

To overcome these challenges, sorters must deliver:

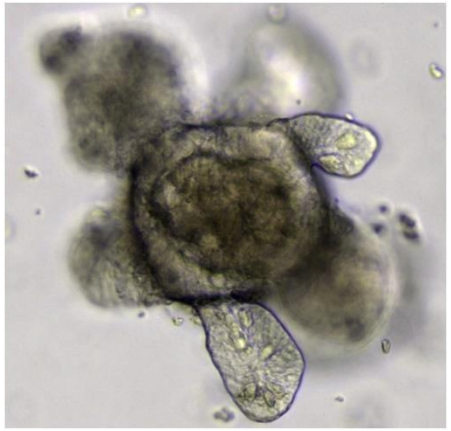
- ✅ Droplet Stability
- ✅ High Throughput
- ✅ Accurate Detection
- ✅ Purity of Sorted Samples
- ✅ Maximized Recovery

SONY Solution: Large Particle Sorting Module using 130 µm nozzle

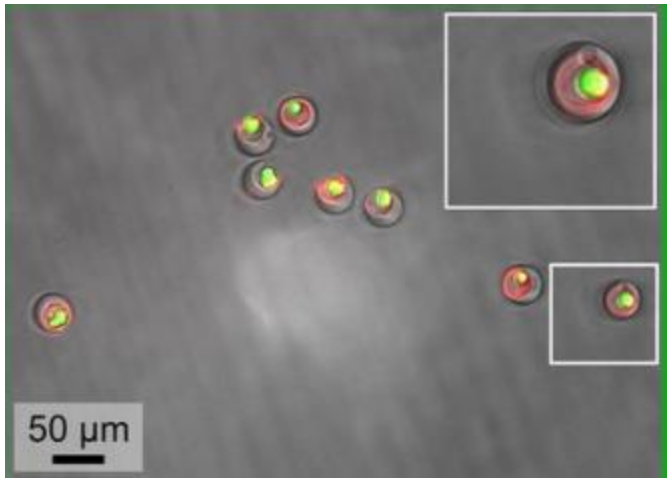
Large Particle Sorting Applications



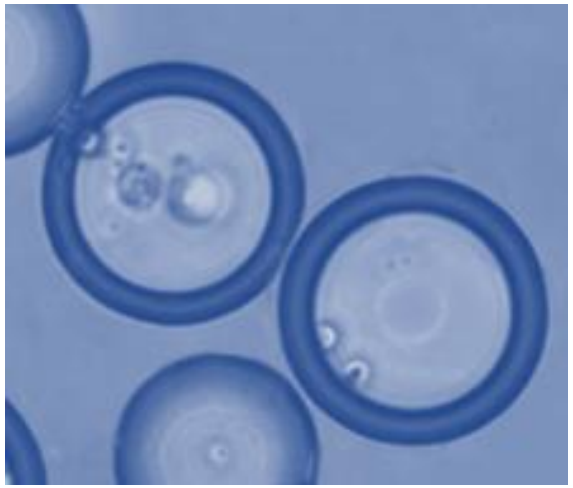
Large cells



Organoids



Hydrogel microcarriers



Double - emulsion drops

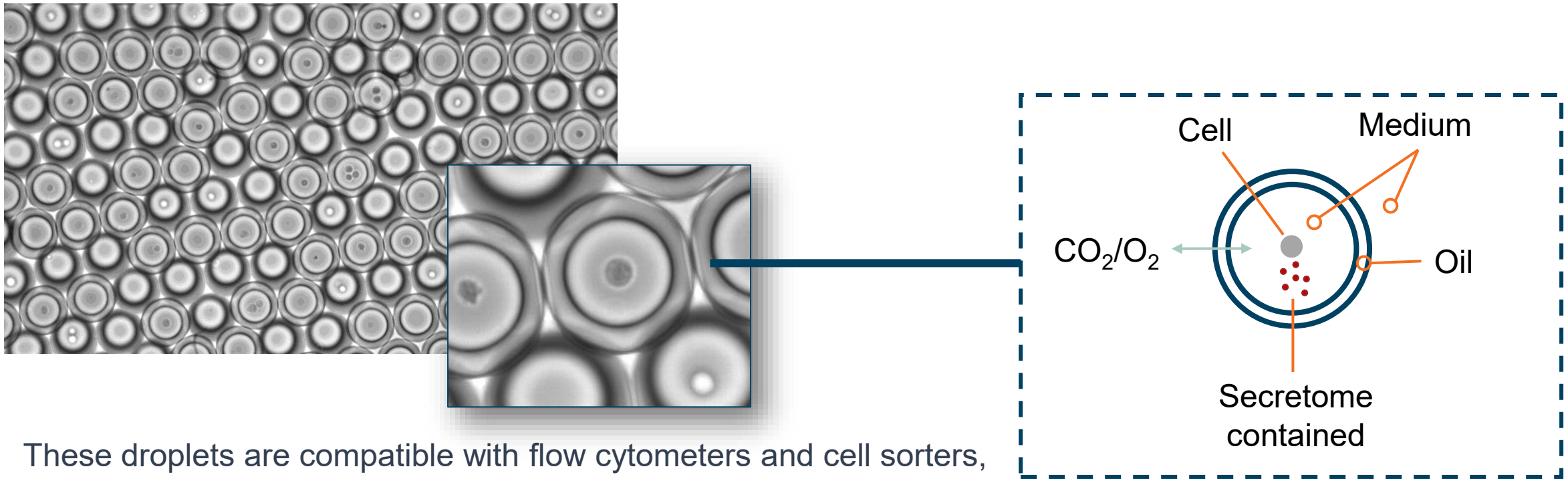
Oversized and irregularly shaped cells such as **tumor cells and megakaryocytes** play critical roles in disease progression and efficient sorting enables deeper investigations into their functions and interactions.

Double emulsions drops and hydrogel particles enable high-throughput single-cell metabolomics and genomic analysis by providing a compartmentalization system that can be used for a variety of applications.

Sorting Double Emulsion Droplets using Sony Cell Sorter

SONY

DE droplets are highly monodispersed and act as individual microreactors

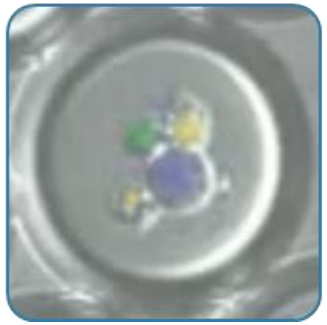
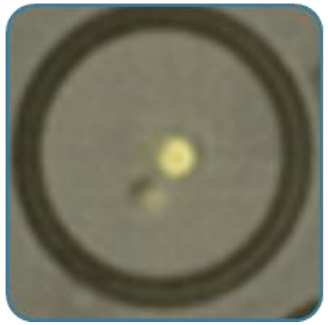
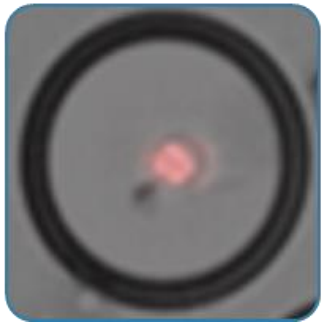
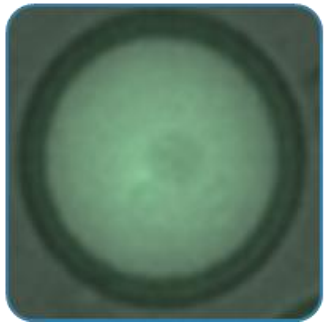
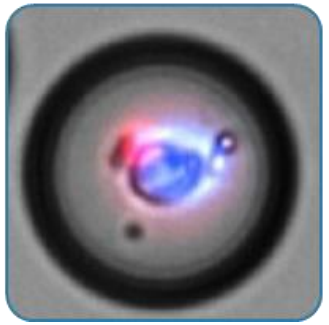
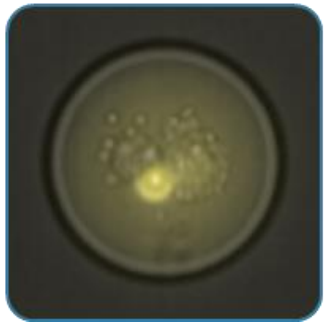
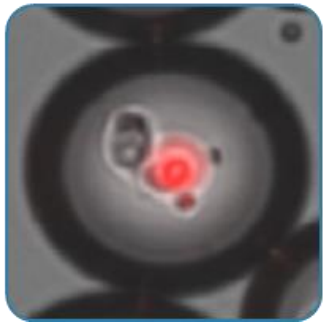


These droplets are compatible with flow cytometers and cell sorters, allowing seamless integration with existing gold-standard technologies.

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Applications using Double Emulsion Droplets

SONY

Cell-cell interactions	Labelling of **dead cells**	Labelling of **cells**	Release of **granzyme B**	Release of **antibodies**	Release of **cytokines**	**Caspase** activity
						

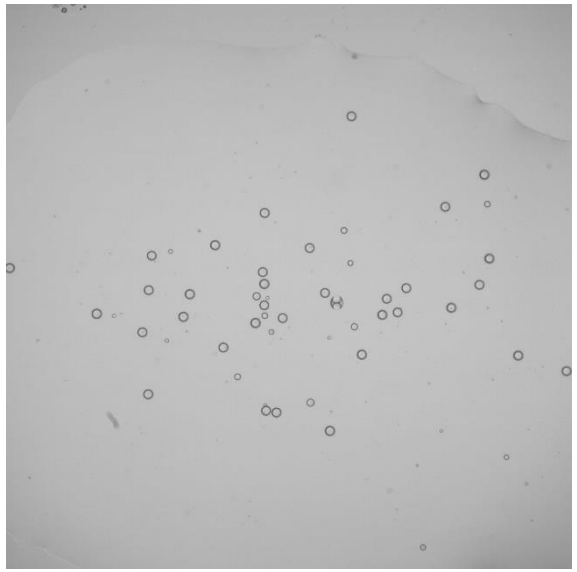
It may also be combined with staining of cell surface markers like in traditional cytometry analysis

Sorting Samplix DE50 for enhanced recovery

SONY

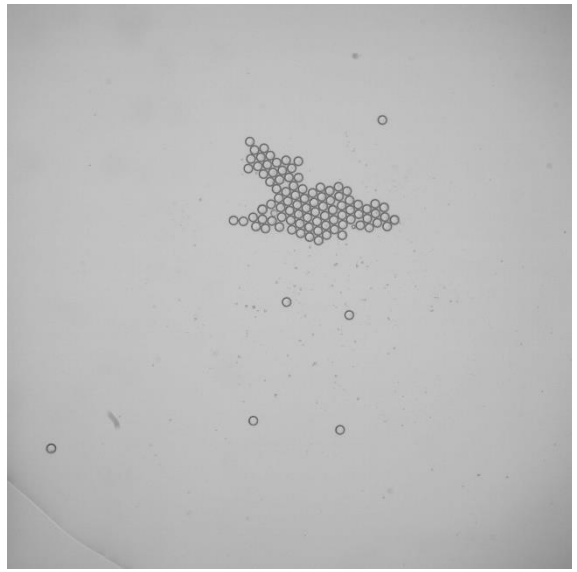
The new large particle option was used to sort DE50 particles from Samplix. 100 droplets of a cell-killing assay were sorted on a glass slide

Without large particle sorting option



~45 DE50s counted in total

With new large particle sorting option



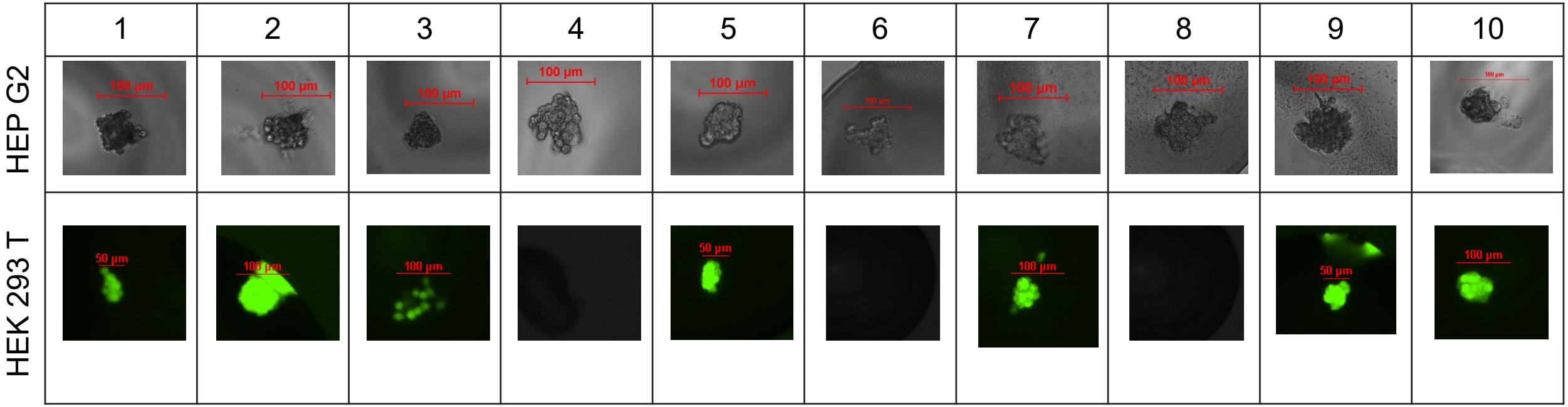
~97 DE50s counted in total

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Sorting Spheroids using MA900 Cell Sorter

SONY

Spheroids formed using HEPG2 and HEK293T cells were sorted using 130 µm microfluidics chip using MA900 Cell Sorter using the large particle option. Recovery of the spheroids was evaluated using microcopy.



Purity: number of wells with a single spheroid / 96 well
Recovery: number of spheroids sorted / 96 well

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Large Particle Software Options

1. Autosetup for 25–35 µm Particles -
   Automated droplet delay adjustments create stable droplet formation, enabling higher recovery and reliability when working with semi-large particles.
2. Particle Size Selection -
   Advanced trigger settings prevent misclassification, ensuring accurate detection and gating of complex particles such as organoids and spheroids.
3. Recovery Sort Mode -
   Flexible settings allow capture of droplets before and after a target event, maximizing yield with user-controlled balance between recovery and purity.
4. Priority Sort -
   Define target and non-target events to minimize unnecessary aborts, boosting recovery when mixed encapsulations do not compromise experimental outcomes.

Large Particle Software Options

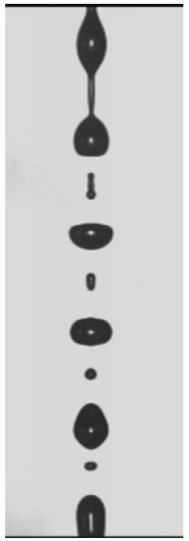
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   Flexible settings allow capture of droplets before and after a target event, maximizing yield with user-controlled balance between recovery and purity.
4. Priority Sort -
   Define target and non-target events to minimize unnecessary aborts, boosting recovery when mixed encapsulations do not compromise experimental outcomes.

1. Auto Calibration for 25 – 35 µm particles

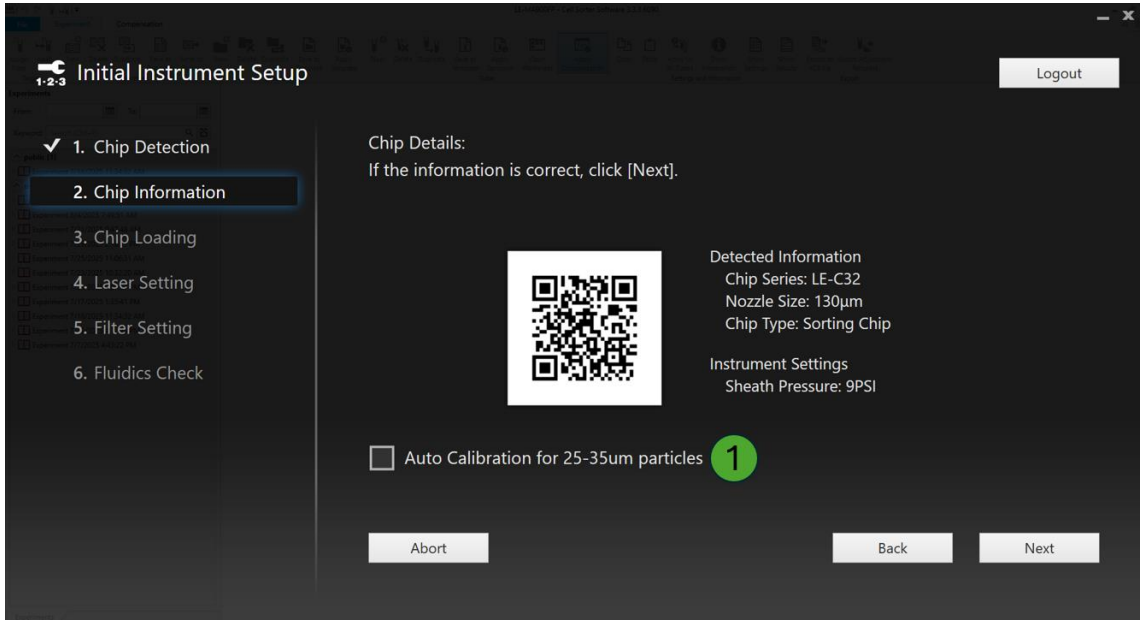
- Large particle disrupt the break off point (BOP) of the sort stream resulting in unstable droplet formation
- New Auto Calibration Algorithm for 25-35 µm particles adjusts instrument BOP settings to reduce interference caused by large particles resulting in stable droplets for optimal sorting



Typical influence of large particle on droplet breakoff



Stable Sorting Stream with New Autosetup Algorithm

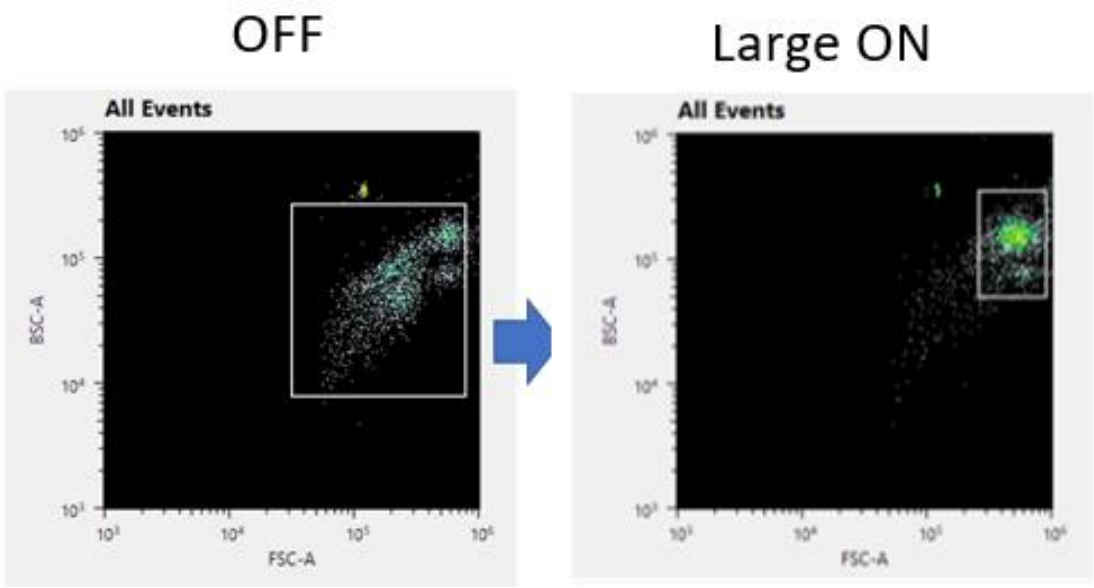


Large Particle Software Options

1. Autosetup for 25–35 µm Particles -
   Automated droplet delay adjustments create stable droplet formation, enabling higher recovery and reliability when working with semi-large particles.
2. Particle Size Selection -
   Advanced trigger settings prevent misclassification, ensuring accurate detection and gating of complex particles such as organoids and spheroids.
3. Recovery Sort Mode -
   Flexible settings allow capture of droplets before and after a target event, maximizing yield with user-controlled balance between recovery and purity.
4. Priority Sort -
   Define target and non-target events to minimize unnecessary aborts, boosting recovery when mixed encapsulations do not compromise experimental outcomes.

2. Size Selection

- Large and heterogeneous particles typically have unstable FSC signatures
- Size selection (advanced trigger settings) prevent misclassification → accurate detection and gating of complex particles such as organoids and spheroids
- Result: Particle is registered as one event instead of multiple
- Aids in visualization, gating, and assigning fluorescence signatures to proper particles
- Custom settings for Normal (<25 µm), Semi-large (25-35 µm), Large (35-50 µm), Very large (>50 µm)



Large Particle Software Options

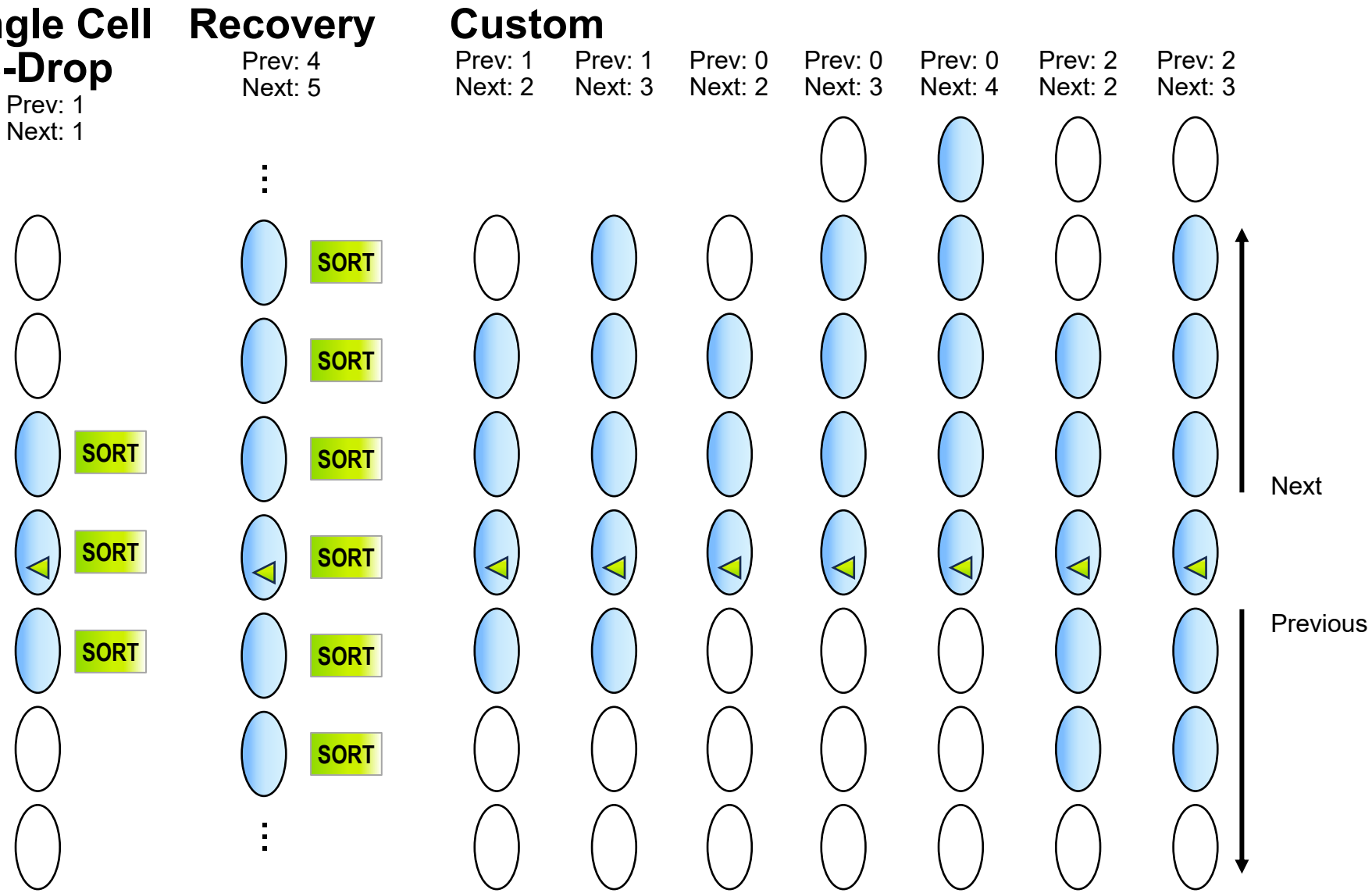
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3. Recovery Sort Mode

SONY

RECOVERY MODE

- Used for large particle sorting
- An extension of Single Cell 3-Drop sort mode, with a possibility to adjust the range of droplets to be sorted
- Sorts a wider range of droplets to account for droplet formation instability
- The range of droplets can be adjusted



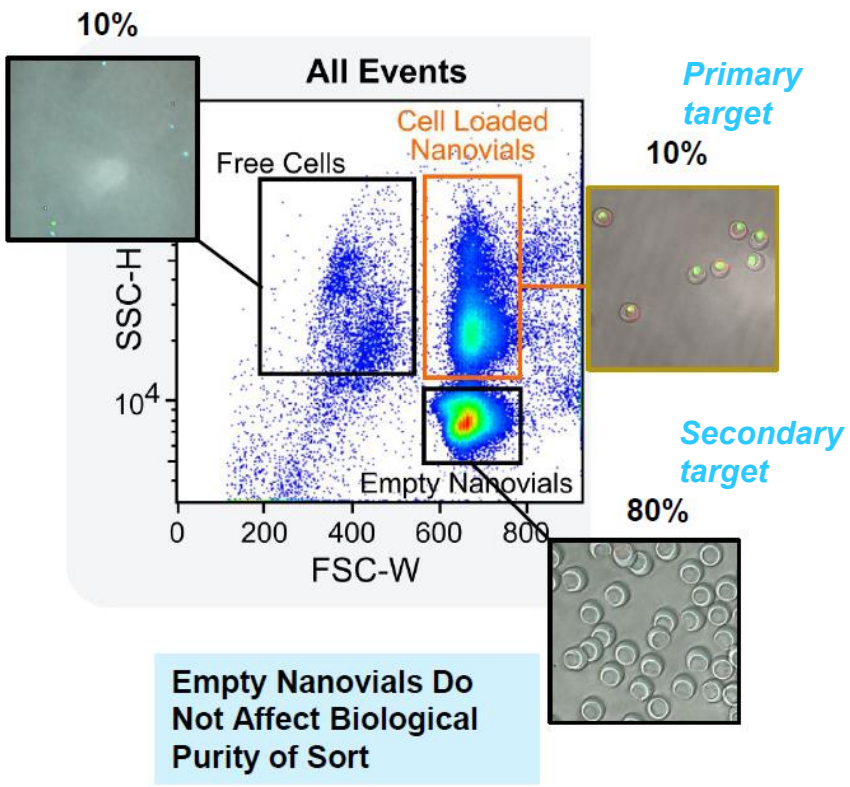
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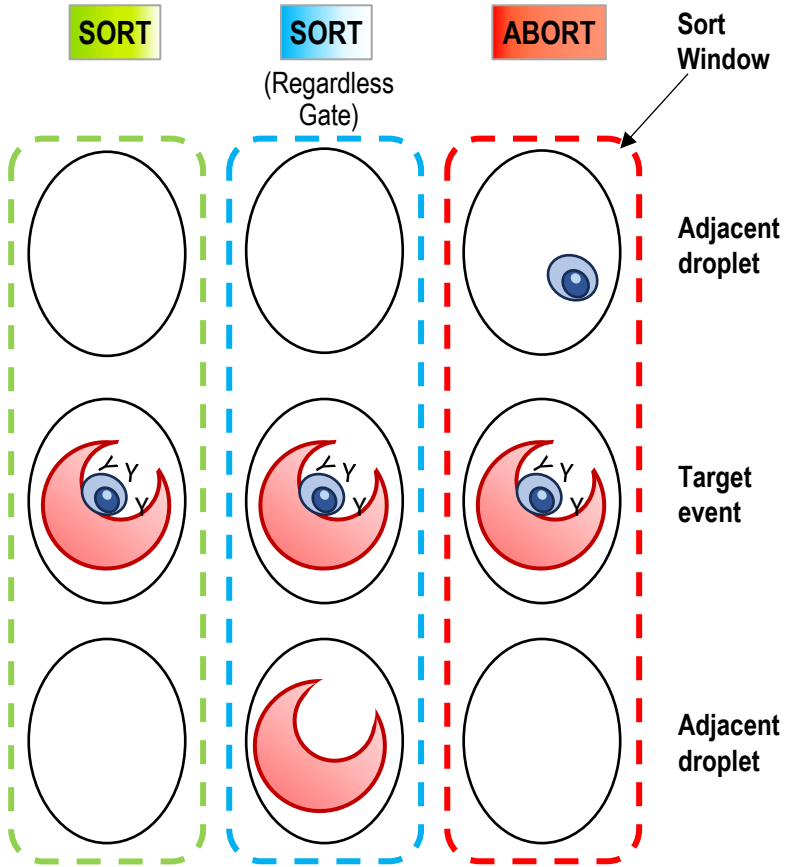
4. Priority Option: Single Cell (3 drop) or Recovery mode

SONY

- Minimizes aborts when using Single Cell (3drop) or Recovery sort modes
- Typically, sort events would be aborted when non-targets or targets are in proximity of sort windows
- Priority mode allows for the designation of ‘primary’ and ‘secondary’ targets. When ‘secondary’ targets are detected within proximity of ‘primary’ sort windows, the sort event is sorted into the ‘primary’ tube instead of being aborted

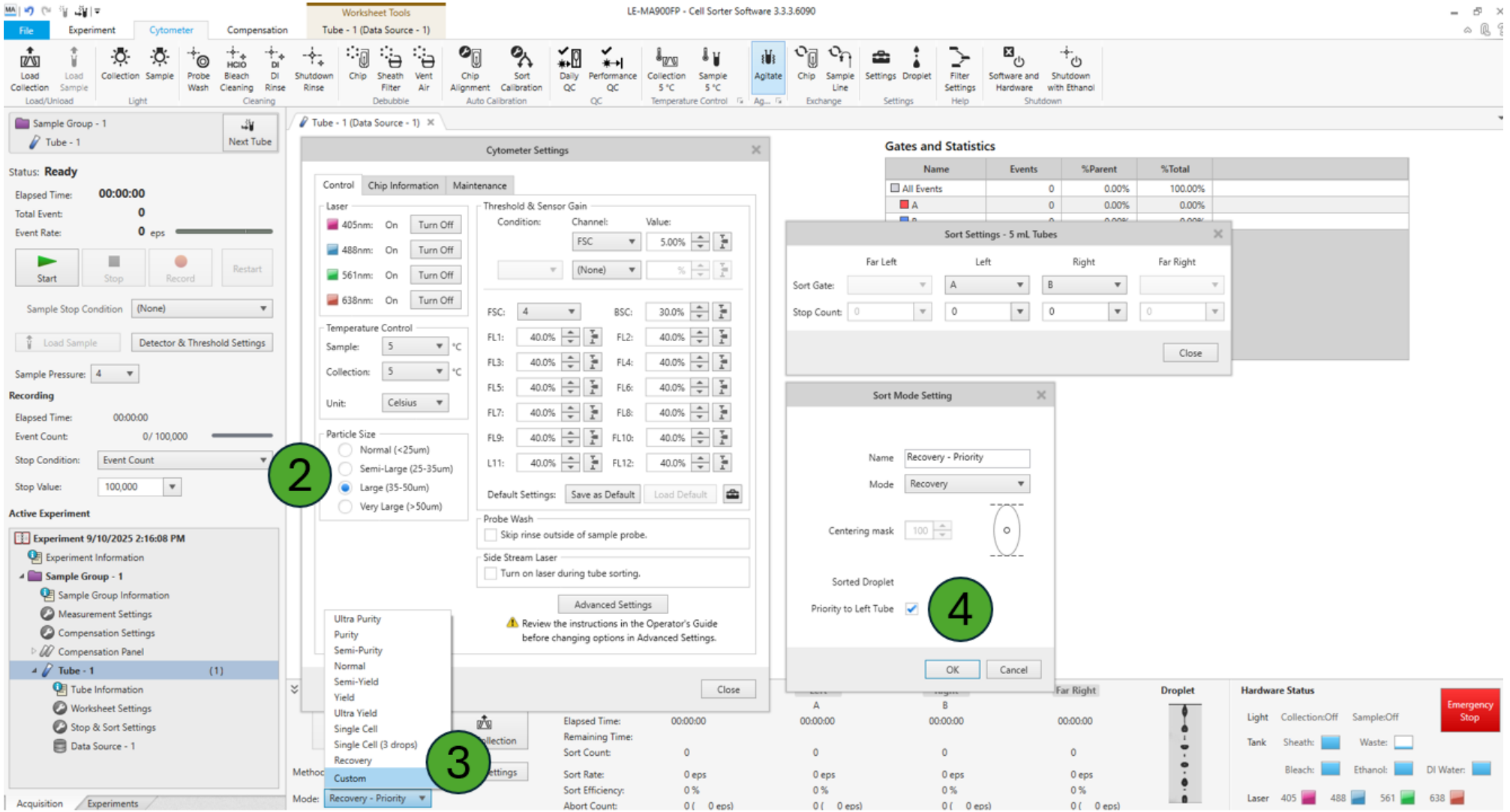


Single Cell sort mode + Regardless Gate



Event not considered in sort logic. Reduce abort rates and achieve up to 5x increase in sort rate while maintaining sort efficiency

Software Overview



SH800 TRAINING

Questions?